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Species without borders: the diversification of giant danios in Sri Lanka (Teleostei, Danionidae, *Devario*)

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Abstract

The five species of *Devario* in Sri Lanka form a monophyletic insular diversification. *Devario memorialis*, *D. micronema* and *D. pathirana* are specialists that inhabit rainforest foothill streams, while *D. altus* **sp. nov.**, is confined to a single montane site (1580 m asl). Finally, *D. malabaricus*, a generalist species associated with open lentic and lotic habitats, is widely distributed from ~0–1500 m asl. The taxonomy of these species has been handicapped by a high degree of intraspecific phenetic variation. Here we construct a phylogeny of, and haplotype networks for, Sri Lankan *Devario* using a molecular dataset derived from the *cox1* and *cytb* mitochondrial DNA markers and the *rag1* and *irdp* nuclear DNA markers representative of all five species. We compare the mitochondrial and nuclear phylogenies for evidence of discordance, showing that *D. malabaricus* appears to have hybridized with all the other species, except *D. memorialis*, in their contact zones. The phenotype of *D. malabaricus* varies with elevation as a cline, partly converging on the phenotype of *D. altus* **sp. nov.**, possibly through introgression with *D. altus* **sp. nov.** or adaptation to hill-stream habitats. Through a morphological analysis of 363 voucher specimens representative of all five species, we characterize the species, thereby stabilizing their taxonomy.

Key Words

Cypriniformes, Danio, new species, mitonuclear discordance, phylogeny, phylogeography, systematics, taxonomy

Introduction

The present-day island of Sri Lanka is separated from India by the ~10 m deep, 30 km wide Palk Strait. Successive sea-level low-stands since the Oligocene saw this shallow-shelf sea being bridged by the Palk Isthmus which, at the Last Glacial Maximum, was almost 200 km wide (Pethiyagoda and Sudasinghe 2021). Except for short periods, the Isthmus was emergent for most of the past 5 My, providing a corridor for biotic exchange between Sri Lanka and the mainland (Pethiyagoda and Sudasinghe 2021). Notwithstanding this terrestrial connectivity, however, Sri Lanka exhibits remarkable biotic insularity vis-à-vis India (Bossuyt et al. 2004). Pethiyagoda and Sudasinghe (2021) concluded that for most of the time the Palk Isthmus was emergent since the Miocene, rainfall was too strongly seasonal to support the exchange of species dependent on a perhumid climate.

The tropical Asian freshwater-fish genus *Devario* (Cyprinidae: Danionidae) comprises some 44 valid species ranging from Sri Lanka through the Indian Subcontinent and Myanmar to Southeast Asia and southern China (Fric-

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ke et al. 2025). Known in the popular literature as giant danios, these small, brightly coloured fishes are ubiquitous in the region's freshwaters, usually numbering among those most frequently encountered in streams and rivers.

The recent literature recognizes seven valid species of *Devario* from Sri Lanka: *D. malabaricus* (Jerdon, 1849), *D. micronema* (Bleeker, 1863a), *D. pathirana* (Kottelat & Pethiyagoda, 1990), *D. memorialis* Sudasinghe, Pethiyagoda & Meegaskumbura, 2020a, and three species described by Batuwita et al. (2017): *D. annnataliae*, *D. udenii* and *D. monticola*. The last name was proposed by Batuwita et al. (2017) as a replacement name for *Danio lineolatus* Bleeker, 1863a, which is a secondary homonym in *Devario* of *Leuciscus lineolatus* Blyth, 1858. Here we show that *D. annnataliae* and *D. udenii* are synonyms of *D. micronema*, while *D. monticola* is a synonym of *D. malabaricus*. We also describe, from the highlands of Sri Lanka, *Devario altus*, a new species. Furthermore, *D. lineolatus* and *D. micronema* benefited from only brief original descriptions that are insufficient to distinguish them from each other (Sudasinghe and Pethiyagoda 2019). Their types have recently been rediscovered, allowing us to clarify their identity (Sudasinghe et al. 2025).

All species of *Devario* exhibit a coloration consisting of stripes, bars and blotches on the sides of the body. While some aspects of coloration remain consistent within species, others show considerable variation. To be useful as a taxonomic character, coloration needs to be characterized rigorously, as proposed, for example, by Fang (1998). Furthermore, the ubiquity of *Devario* in Sri Lankan freshwaters suggests that the various species encounter one another in contact zones, with potential for hybridization. Hybrid individuals, in turn, risk causing taxonomic confusion. Signals of hybridization in *Devario* in the wild, however, have been reported in only a few studies (e.g., Kullander et al. 2017; Sudasinghe et al. 2020a).

All but one of the five valid species of *Devario* on Sri Lanka are endemic to the rainforests of the island's perhumid south-western 'wet zone' (rainfall > 2.5 m/y, minimum monthly rainfall > 0.1 m). Three of them are each confined to a single river basin: *D. pathirana* to the Nilwala, *D. memorialis* to the Ma, and *D. altus* sp. nov. to the upper reaches of the Mahaweli, > 1500 m asl (Batuwita et al. 2017; Sudasinghe and Pethiyagoda 2019; Sudasinghe et al. 2020a). *Devario micronema* is confined to shaded streams draining lowland rainforests from the Kelani to the Gin River basin, while the fifth species, *D. malabaricus*, ranges widely through southern India and Sri Lanka (Sudasinghe et al. 2020a).

Sudasinghe et al. (2020a) showed the Sri Lankan diversification of *Devario* to be monophyletic, having occurred in the Plio-Pleistocene. They also showed that gene flow between the Indian and Sri Lankan populations of *D. malabaricus* had continued until the late Pleistocene; that *D. micronema* and *D. pathirana* have a sister-group relationship, with a Pleistocene crown age; and that {*D. micronema*+*D. pathirana*} diverged from its sister group, {*D. memorialis*}, in the Pliocene. Additionally, they recovered a sister-group relationship between *D. malabaricus* and the population here described as *D. altus* sp. nov., with a divergence dating to the Pleistocene.

This raises the question of whether *Devario altus* sp. nov. is a relictual narrow-range endemic that survived the near-total deforestation of the Sri Lankan highlands for plantation agriculture; or whether it is a population of *D. malabaricus* that exhibits a distinct phenotype as a result of local adaptation to the highland environment.

Here, through a phylogenetic analysis based on two mitochondrial and two nuclear DNA markers, we investigate the relationships of Sri Lankan *Devario* by generating and comparing their mitochondrial and nuclear phylogenies; constructing a phylogenetic network of the species; calculating their inter- and intraspecific genetic distances; and investigating their genetic diversity. We also compare the nuclear and mitochondrial phylogenies for evidence of discordance, seeking to detect evidence of hybridization or incomplete lineage sorting. Furthermore, we conduct a detailed morphological analysis based on an extensive series of preserved specimens of Sri Lankan *Devario* across the range of the genus on the island. We also evaluate phenotypic variation within and between the five species in the light of the molecular phylogenies. Finally, we review and clarify the taxonomy of Sri Lankan *Devario*, provide diagnoses for the species we consider to be valid, describe a new species, and provide a key to their identification.

Material and methods

Fieldwork

Fieldwork in Sri Lanka was conducted with permission from the Department of Wildlife Conservation (permit no. WL/3/2/59/14) and the Forest Department (permit no. R&E/RES/NFSRCM/14-16-4) granted to HS and MM. The ethics associated with specimen collection, euthanization (using tricaine methanesulfonate), tissue sampling, and specimen fixation were approved by the Postgraduate Institute of Science, University of Peradeniya. A total of 136 samples from 65 different locations covering the entire geographic range of *Devario* in Sri Lanka were collected and utilized for the molecular analysis (Table 1).

Taxon definition

Sudasinghe et al. (2020a) recognized five species of *Devario* in Sri Lanka: *D. malabaricus*, *D. micronema*, *D. memorialis*, *D. pathirana*, and the one we now describe as *D. altus* sp. nov., from Bopathalawa, 1580 m asl. This represents the elevational limit for native freshwater fishes in Sri Lanka, otherwise reached only by *Garra ceylonensis* Bleeker, 1863a and *Schistura notostigma* (Bleeker, 1863b) (Pethiyagoda and Sudasinghe 2021). Some populations of *Devario* in the central hills, however, contain individuals that exhibit a color pattern similar to that of

D. altus sp. nov. but these contain a mix of mitochondrial haplotypes associated with *D. altus* sp. nov. and *D. malabaricus*. They are here referred to as '*D.* cf. *malabaricus* (highland phenotype)'.

Additionally, several sampling sites in the southwestern wet zone contained individuals that show mitonuclear discordance between *D. malabaricus* and *D. micronema*, and *D. malabaricus* and *D. pathirana*, while exhibiting phenotypic characters that are intermediate between the respective pairs. These specimens are assumed to be hybrids.

DNA protocols

Gene nomenclature follows the ZFIN Zebrafish Nomenclature Conventions (https://goo.gl/MdawKQ). The study of Sudasinghe et al. (2020a) generated sequences for Sri Lankan *Devario* from a sampling of 27 locations: 53 partial mitochondrial cytochrome c oxidase subunit 1 (*cox1*) (GenBank MN005700–MN005752), 52 partial mitochondrial cytochrome b (*cytb*) (GenBank MN005753–MN005804), and 12 nuclear recombination activating protein 1, exon 3 (*rag1*) (GenBank MN005805–MN005816). In the present study, we generated for the first time an additional 83 *cox1* (GenBank PQ842727–PQ842809), 83 *cytb* (GenBank PQ848127–PQ848209), 95 *rag1* (GenBank PQ848269–PQ848363), and 59 nuclear interphotoreceptor retinoid-binding protein 2 (*irbp*) sequences (GenBank PQ848210–PQ848268) (Table 1). This total dataset is derived from 136 specimens occurring at 65 locations encompassing the entire geographic range of *Devario* on the island (Fig. 1).

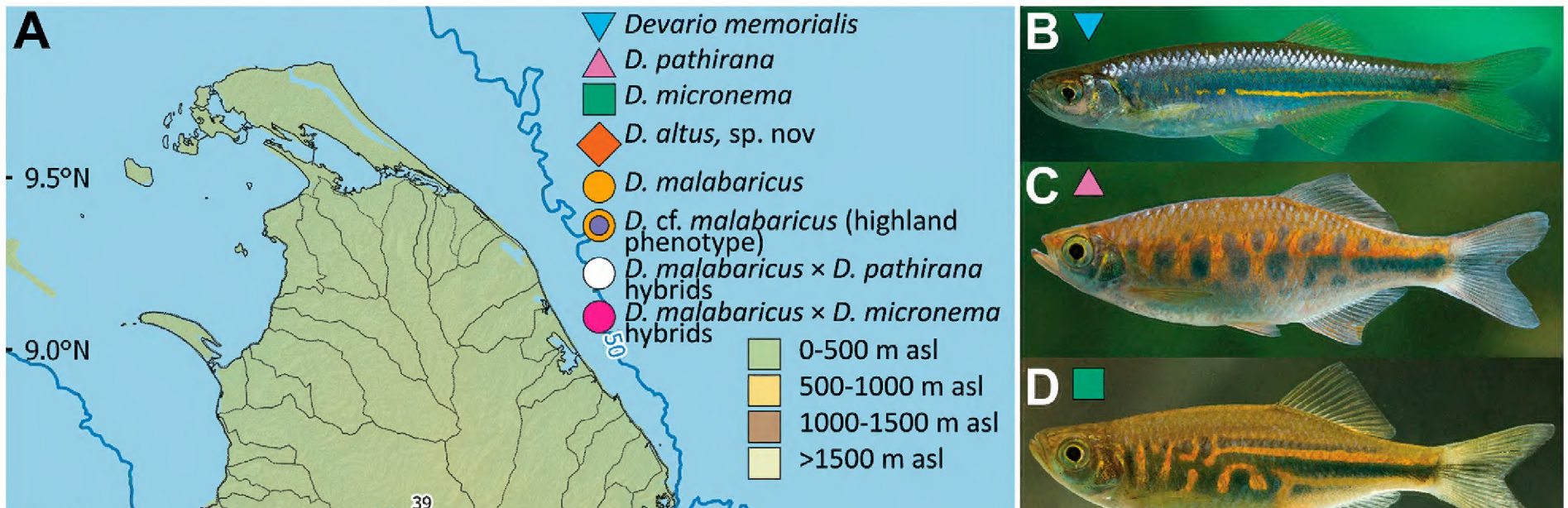

Figure 1. A. Sampling localities for Sri Lankan *Devario* for the molecular analysis in the present study. Numbers on the map represent the sampling localities in Table 1. Major river-basin boundaries are marked with narrow black lines. Live coloration of **B.** *D. memorialis*; **C.** *D. pathirana*; **D.** *D. micronema*; **E.** *D. altus*, sp. nov.; **F.** *D. malabaricus*. Specimen coloration of populations of *Devario* identified as **G.** *D.* cf. *malabaricus* (highland phenotype); **H.** *D. malabaricus* × *D. pathirana* hybrids; **I.** *D. malabaricus* × *D. micronema* hybrids.

Table 1. Details of sampling localities of species of *Devario* for the molecular analysis. Index represent sampling localities as represented in the map of Fig. 1. Newly generated sequences are in bold.

DZ	Genus\Species	Location	GPS Coordinates	River basin	Elevation (m asl)	*cox1*	*cytb*	*rag1*	*irbp*	Index
DZ1451	*Devario* cf. *malabaricus*	Pambahinna, Belihuloya	6.69688°N, 80.77613°E	Walawe	480	**PQ842727**	**PQ848127**	**PQ848269**	**PQ848210**	1
DZ3031	*Devario malabaricus*	Dellabada, Elapatha, Ratnapura	6.680488°N, 80.34987°E	Kalu	29	MN005714	MN005767	NA	NA	2
DZ3040	*Devario malabaricus*	Kolamunu Oya anicut, Bingiriya	7.603053°N, 79.9502°E	Deduru	24	MN005717	MN005770	NA	NA	3
DZ3043	*Devario malabaricus*	Kadigawa bridge, Deduru Oya	7.697535°N, 79.99817°E	Deduru	27	MN005718	MN005771	NA	NA	4
DZ3139	*Devario memorialis*	Pannalthota, Uggoda, Aranayake	7.142825°N, 80.46855°E	MaaOya	236	MN005734	MN005787	**PQ848270**	**PQ848211**	5
DZ3140	*Devario memorialis*	Pannalthota, Uggoda, Aranayake	7.142825°N, 80.46855°E	MaaOya	236	MN005735	MN005788	MN005816	**PQ848212**	5
DZ3147	*Devario memorialis*	Pannalthota, Uggoda, Aranayake	7.142825°N, 80.46855°E	MaaOya	236	MN005736	MN005789	**PQ848271**	**PQ848213**	5
DZ3169	*Devario malabaricus*	Amban River at Swarnapaligama, Polonnaruwa	7.862102°N, 80.95588°E	Mahaweli	58	MN005705	MN005758	MN005805	NA	6
DZ3174	*Devario malabaricus*	Below Dora-dahaya-sorowwa, Polonnaruwa	7.871488°N, 80.96782°E	Mahaweli	56	MN005706	MN005759	NA	NA	7
DZ3190	*Devario malabaricus*	Below Dora-dahaya-sorowwa, Polonnaruwa	7.871488°N, 80.96782°E	Mahaweli	56	MN005707	MN005760	NA	NA	7
DZ3197	*Devario* cf. *malabaricus*	Agrapatana	6.864361°N, 80.70317°E	Mahaweli	1380	MN005700	MN005753	MN005809	NA	8
DZ3198	*Devario* cf. *malabaricus*	Agrapatana	6.864361°N, 80.70317°E	Mahaweli	1380	MN005701	MN005754	**PQ848272**	NA	8
DZ3219	*Devario malabaricus*	Miyanawita, Deraniyagala	6.894867°N, 80.3549°E	Kelani	104	**PQ842728**	**PQ848128**	**PQ848273**	NA	9
DZ3220	*Devario malabaricus*	Miyanawita, Deraniyagala	6.894867°N, 80.3549°E	Kelani	104	**PQ842729**	**PQ848129**	NA	NA	9
DZ3273	*Devario malabaricus*	Sarasavi Oya, Peradeniya	7.257456°N, 80.59572°E	Mahaweli	481	MN005708	MN005761	NA	NA	10
DZ3275	*Devario malabaricus*	Sarasavi Oya, Peradeniya	7.257456°N, 80.59572°E	Mahaweli	481	MN005709	MN005762	MN005807	NA	10
DZ3288	*Devario malabaricus*	Remuna, Horana	6.685759°N, 80.06498°E	Kalu	17	MN005715	MN005768	**PQ848274**	NA	11
DZ3289	*Devario malabaricus*	Remuna, Horana	6.685759°N, 80.06498°E	Kalu	17	MN005716	MN005769	NA	NA	11
DZ3307	*Devario malabaricus*	Indipelagoda, Pitigala	6.3341°N, 80.22993°E	Bentara	18	MN005719	MN005772	NA	NA	12
DZ3316	*Devario pathirana*	Kosgahadola ela, Mulatiyana	6.17874°N, 80.58203°E	Nilwala	162	MN005751	MN005803	MN005815	NA	13
DZ3317	*Devario pathirana*	Kosgahadola ela, Mulatiyana	6.17874°N, 80.58203°E	Nilwala	162	MN005752	MN005804	**PQ848275**	**PQ848214**	13
DZ3326	*Devario micronema*	Rukmale-dola, Pallegama, Deniyaya	6.34131°N, 80.53855°E	Gin	376	MN005702	MN005755	**PQ848276**	**PQ848215**	14
DZ3327	*Devario micronema*	Rukmale-dola, Pallegama, Deniyaya	6.34131°N, 80.53855°E	Gin	376	MN005703	MN005756	MN005814	**PQ848216**	14
DZ3328	*Devario micronema*	Kiriwel-dola, Deniyaya	6.3615°N, 80.52483°E	Gin	315	MN005704	MN005757	**PQ848277**	**PQ848217**	15
DZ3336	*Devario malabaricus* × *pathirana* hybrid	Suriyakanda	6.45063°N, 80.61684°E	Walawe	1009	**PQ842730**	**PQ848130**	**PQ848278**	NA	16
DZ3337	*Devario malabaricus* × *pathirana* hybrid	Suriyakanda	6.45063°N, 80.61684°E	Walawe	1009	**PQ842731**	**PQ848131**	**PQ848279**	NA	16
DZ3351	*Devario micronema*	Belipola, Delgoda, Kalawana	6.49355°N, 80.40451°E	Kalu	227	MN005742	MN005795	NA	NA	17
DZ3352	*Devario micronema*	Belipola, Delgoda, Kalawana	6.49355°N, 80.40451°E	Kalu	227	MN005743	MN005796	NA	NA	17
DZ3353	*Devario micronema*	Malwara FR, Halwala, near Kalawana	6.54075°N, 80.38239°E	Kalu	287	MN005744	MN005797	NA	NA	18
DZ3354	*Devario micronema*	Malwara FR, Halwala, near Kalawana	6.54075°N, 80.38239°E	Kalu	287	MN005745	MN005798	NA	NA	18
DZ3363	*Devario micronema*	Athwelthota	6.5382°N, 80.2901°E	Kalu	81	MN005746	MN005799	MN005813	NA	19
DZ3364	*Devario micronema*	Athwelthota	6.5382°N, 80.2901°E	Kalu	81	MN005747	MN005800	NA	NA	19
DZ3379	*Devario* cf. *malabaricus*	Mouth of Belihul Oya to Samanala Reservoir, Pambahinna	6.69388°N, 80.77855°E	Walawe	465	**PQ842732**	**PQ848132**	**PQ848280**	**PQ848218**	20
DZ3380	*Devario* cf. *malabaricus*	Mouth of Belihul Oya to Samanala Reservoir, Pambahinna	6.69388°N, 80.77855°E	Walawe	465	**PQ842733**	**PQ848133**	**PQ848281**	**PQ848219**	20
DZ3390	*Devario malabaricus*	Hathbamba ella, near Niriella, Ratnapura	6.6339°N, 80.35778°E	Kalu	87	**PQ842734**	**PQ848134**	**PQ848282**	NA	21
DZ3391	*Devario malabaricus*	Hathbamba ella, near Niriella, Ratnapura	6.6339°N, 80.35778°E	Kalu	87	**PQ842735**	**PQ848135**	**PQ848283**	NA	21

DZ	Genus\Species	Location	GPS Coordinates	River basin	Elevation (m asl)	*cox1*	*cytb*	*rag1*	*irbp*	Index
DZ3401	*Devario malabaricus*	Murugan Oya, Kosgahakanda, close to Miyanawita, Deraniyagala	6.87898°N, 80.36303°E	Kelani	171	**PQ842736**	**PQ848136**	**PQ848284**	NA	22
DZ3402	*Devario malabaricus*	Murugan Oya, Kosgahakanda, close to Miyanawita, Deraniyagala	6.87898°N, 80.36303°E	Kelani	171	**PQ842737**	**PQ848137**	**PQ848285**	NA	22
DZ3403	*Devario malabaricus*	Kahanawita Ela, enroute to Deraniyagala	6.965001°N, 80.28865°E	Kelani	96	**PQ842738**	**PQ848138**	**PQ848286**	NA	23
DZ3404	*Devario malabaricus*	Kahanawita Ela, enroute to Deraniyagala	6.965001°N, 80.28865°E	Kelani	96	**PQ842739**	**PQ848139**	**PQ848287**	NA	23
DZ3405	*Devario malabaricus* × *micronema* hybrid	Andawal-dola, near Udagama waterfall, Padukka	6.8237°N, 80.16152°E	Kalu	47	**PQ842740**	**PQ848140**	**PQ848288**	NA	24
DZ3406	*Devario malabaricus* × *micronema* hybrid	Andawal-dola, near Udagama waterfall, Padukka	6.8237°N, 80.16152°E	Kalu	47	**PQ842741**	**PQ848141**	**PQ848289**	NA	24
DZ3425	*Devario* cf. *malabaricus*	Nawagamu Oya, Kobonilla	7.352957°N, 80.85151°E	Mahaweli	850	**PQ842742**	**PQ848142**	**PQ848290**	**PQ848220**	25
DZ3426	*Devario* cf. *malabaricus*	Nawagamu Oya, Kobonilla	7.352957°N, 80.85151°E	Mahaweli	850	**PQ842743**	**PQ848143**	**PQ848291**	NA	25
DZ3439	*Devario malabaricus*	Suduganga, Naula, Matale	7.51782°N, 80.64314°E	Mahaweli	328	MN005710	MN005763	**PQ848292**	NA	26
DZ3504	*Devario malabaricus*	Badulu Oya, Kandeketiya	7.18305°N, 81.00393°E	Mahaweli	123	MN005711	MN005764	NA	NA	27
DZ3509	*Devario* cf. *malabaricus*	Close to Minipe	7.20084°N, 80.97425°E	Mahaweli	114	**PQ842744**	**PQ848144**	**PQ848293**	**PQ848221**	28
DZ3510	*Devario* cf. *malabaricus*	Close to Minipe	7.20084°N, 80.97425°E	Mahaweli	114	**PQ842745**	**PQ848145**	**PQ848294**	NA	28
DZ3528	*Devario malabaricus*	Heen Ganga, Sulugune, Wilgamuwa	7.46893°N, 80.90102°E	Mahaweli	139	MN005712	MN005765	**PQ848295**	NA	29
DZ3529	*Devario malabaricus*	Heen Ganga, Sulugune, Wilgamuwa	7.46893°N, 80.90102°E	Mahaweli	139	MN005713	MN005766	NA	NA	29
DZ3612	*Devario malabaricus*	Downstream of Asupini falls, Aranayake	7.12993°N, 80.47746°E	MaaOya	266	MN005720	MN005773	MN005806	NA	30
DZ3613	*Devario malabaricus*	Downstream of Asupini falls, Aranayake	7.12993°N, 80.47746°E	MaaOya	266	MN005721	MN005774	**PQ848296**	**PQ848222**	30
DZ3614	*Devario malabaricus*	Downstream of Asupini falls, Aranayake	7.12993°N, 80.47746°E	MaaOya	266	MN005722	MN005775	**PQ848297**	NA	30
DZ3615	*Devario micronema*	Nakiya-dola, Dickhena	6.46784°N, 80.31908°E	Kalu	125	MN005740	MN005793	MN005812	NA	31
DZ3616	*Devario micronema*	Nakiya-dola, Dickhena	6.46784°N, 80.31908°E	Kalu	125	MN005741	MN005794	NA	NA	31
DZ3688	*Devario malabaricus*	Uduwaka-ella, Dunumana	7.14804°N, 80.20185°E	Kelani	137	MN005723	MN005776	**PQ848298**	NA	32
DZ3689	*Devario malabaricus*	Uduwaka-ella, Dunumana	7.14804°N, 80.20185°E	Kelani	137	MN005724	MN005777	NA	NA	32
DZ3690	*Devario malabaricus*	Malgahawita, Dunumana	7.13089°N, 80.21091°E	AttanagaluOya	84	MN005725	MN005778	**PQ848299**	**PQ848223**	33
DZ3691	*Devario malabaricus*	Malgahawita, Dunumana	7.13089°N, 80.21091°E	AttanagaluOya	84	MN005726	MN005779	NA	NA	33
DZ3694	*Devario malabaricus*	Wahareka	7.07648°N, 80.18678°E	AttanagaluOya	84	MN005727	MN005780	NA	NA	34
DZ3695	*Devario malabaricus*	Wahareka	7.07648°N, 80.18678°E	AttanagaluOya	84	MN005728	MN005781	NA	NA	34
DZ3703	*Devario malabaricus*	Nilgala	7.21837°N, 81.35909°E	Galoya	194	MN005729	MN005782	**PQ848300**	**PQ848224**	35
DZ3704	*Devario malabaricus*	Nilgala	7.21837°N, 81.35909°E	Galoya	194	MN005730	MN005783	NA	NA	35
DZ3738	*Devario* cf. *malabaricus*	Seethagangula, Nallathanni	6.826223°N, 80.52569°E	Kelani	1240	MN005733	MN005786	MN005808	**PQ848225**	36
DZ3739	*Devario* cf. *malabaricus*	Seethagangula, Nallathanni	6.826223°N, 80.52569°E	Kelani	1240	**PQ842746**	**PQ848146**	NA	**PQ848226**	36
DZ3740	*Devario* cf. *malabaricus*	Seethagangula, Nallathanni	6.826223°N, 80.52569°E	Kelani	1240	**PQ842747**	**PQ848147**	**PQ848301**	NA	36
DZ3741	*Devario* cf. *malabaricus*	Madulu oya, udupussellawa	7.059444°N, 80.895°E	Mahaweli	799	**PQ842748**	**PQ848148**	**PQ848302**	NA	37
DZ3742	*Devario* cf. *malabaricus*	Madulu oya, udupussellawa	7.059444°N, 80.895°E	Mahaweli	799	**PQ842749**	**PQ848149**	**PQ848303**	**PQ848227**	37
DZ3743	*Devario* cf. *malabaricus*	Madulu oya, udupussellawa	7.059444°N, 80.895°E	Mahaweli	799	**PQ842750**	**PQ848150**	**PQ848304**	NA	37
DZ3744	*Devario* cf. *malabaricus*	Madulu oya, udupussellawa	7.059444°N, 80.895°E	Mahaweli	799	**PQ842751**	**PQ848151**	**PQ848305**	**PQ848228**	37
DZ3745	*Devario* cf. *malabaricus*	Maa Oya, Hanguranketha	7.19314°N, 80.76513°E	Mahaweli	550	**PQ842752**	**PQ848152**	**PQ848306**	**PQ848229**	38
DZ3746	*Devario* cf. *malabaricus*	Maa Oya, Hanguranketha	7.19314°N, 80.76513°E	Mahaweli	550	**PQ842753**	**PQ848153**	**PQ848307**	NA	38
DZ3747	*Devario* cf. *malabaricus*	Maa Oya, Hanguranketha	7.19314°N, 80.76513°E	Mahaweli	550	**PQ842754**	**PQ848154**	**PQ848308**	**PQ848230**	38
DZ3748	*Devario* cf. *malabaricus*	Maa Oya, Hanguranketha	7.19314°N, 80.76513°E	Mahaweli	550	**PQ842755**	**PQ848155**	NA	NA	38

DZ	Genus\Species	Location	GPS Coordinates	River basin	Elevation (m asl)	*cox1*	*cytb*	*rag1*	*irbp*	Index
DZ3763	*Devario malabaricus*	Kanadara Oya, Thulawelliya, Puleliya, Medawacchiya	8.509139°N, 80.42289°E	MalwathuOya	66	MN005731	MN005784	NA	NA	39
DZ3764	*Devario malabaricus*	Kanadara Oya, Thulawelliya, Puleliya, Medawacchiya	8.509139°N, 80.42289°E	MalwathuOya	66	MN005732	MN005785	NA	NA	39
DZ3857	*Devario micronema*	Makandawa FR, Kitulgala	6.98576°N, 80.40337°E	Kelani	497	MN005737	MN005790	MN005811	NA	40
DZ3858	*Devario micronema*	Makandawa FR, Kitulgala	6.98576°N, 80.40337°E	Kelani	497	MN005738	MN005791	**PQ848309**	**PQ848231**	40
DZ3859	*Devario micronema*	Makandawa FR, Kitulgala	6.98576°N, 80.40337°E	Kelani	497	MN005739	MN005792	**PQ848310**	**PQ848232**	40
DZ3862	*Devario micronema*	Homadola estate, Udugama	6.21576°N, 80.34361°E	Gin	51	MN005748	MN005801	MN005810	NA	41
DZ3863	*Devario micronema*	Homadola estate, Udugama	6.21576°N, 80.34361°E	Gin	51	MN005749	MN005802	**PQ848311**	NA	41
DZ3864	*Devario micronema*	Homadola estate, Udugama	6.21576°N, 80.34361°E	Gin	51	MN005750	NA	**PQ848312**	NA	41
DZ3892	*Devario micronema*	Ma dola, Homadola estate	6.22953°N, 80.36664°E	Gin	93	**PQ842756**	**PQ848156**	**PQ848313**	NA	42
DZ3893	*Devario micronema*	Ma dola, Homadola estate	6.22953°N, 80.36664°E	Gin	93	**PQ842757**	**PQ848157**	**PQ848314**	NA	42
DZ4015	*Devario* cf. *malabaricus*	Rambukpiitya, Nawalapitiya	7.046683°N, 80.5063°E	Mahaweli	604	**PQ842758**	**PQ848158**	**PQ848315**	**PQ848233**	43
DZ4016	*Devario* cf. *malabaricus*	Rambukpiitya, Nawalapitiya	7.046683°N, 80.5063°E	Mahaweli	604	**PQ842759**	**PQ848159**	**PQ848316**	**PQ848234**	43
DZ4166	*Devario* cf. *malabaricus*	Hal Oya, Welimada	6.915981°N, 80.90549°E	Mahaweli	1044	**PQ842760**	**PQ848160**	**PQ848317**	**PQ848235**	44
DZ4167	*Devario* cf. *malabaricus*	Hal Oya, Welimada	6.915981°N, 80.90549°E	Mahaweli	1044	**PQ842761**	**PQ848161**	**PQ848318**	NA	44
DZ4176	*Devario* cf. *malabaricus*	Badulu Oya tributary, Gal-edanda road, Diayatalawa	6.822678°N, 80.93617°E	Mahaweli	1110	**PQ842762**	**PQ848162**	**PQ848319**	**PQ848236**	45
DZ4177	*Devario* cf. *malabaricus*	Badulu Oya tributary, Gal-edanda road, Diayatalawa	6.822678°N, 80.93617°E	Mahaweli	1110	**PQ842763**	**PQ848163**	NA	NA	45
DZ4208	*Devario* cf. *malabaricus*	Hepola Oya, Kuragammala, Bibile	7.174133°N, 81.15335°E	Mahaweli	243	**PQ842764**	**PQ848164**	**PQ848320**	**PQ848237**	46
DZ4209	*Devario* cf. *malabaricus*	Hepola Oya, Kuragammala, Bibile	7.174133°N, 81.15335°E	Mahaweli	243	**PQ842765**	**PQ848165**	**PQ848321**	**PQ848238**	46
DZ4232	*Devario* cf. *malabaricus*	Loggal Oya headwaters, Passara	6.934271°N, 81.14688°E	Mahaweli	844	**PQ842766**	**PQ848166**	**PQ848322**	**PQ848239**	47
DZ4237	*Devario* cf. *malabaricus*	Near Kaluwala, Galbokka, Peraketiya Road, Wellawaya	6.762942°N, 81.0845°E	KirindiOya	299	**PQ842767**	**PQ848167**	**PQ848323**	**PQ848240**	48
DZ4238	*Devario* cf. *malabaricus*	Near Kaluwala, Galbokka, Peraketiya Road, Wellawaya	6.762942°N, 81.0845°E	KirindiOya	299	**PQ842768**	**PQ848168**	**PQ848324**	**PQ848241**	48
DZ4345	*Devario micronema*	Near Udagamkanda, along Udagama road to Wewila	6.816037°N, 80.17039°E	Kalu	157	**PQ842769**	**PQ848169**	**PQ848325**	**PQ848242**	49
DZ4346	*Devario micronema*	Near Udagamkanda, along Udagama road to Wewila	6.816037°N, 80.17039°E	Kalu	157	**PQ842770**	**PQ848170**	**PQ848326**	**PQ848243**	49
DZ4476	*Devario pathirana*	Ugudu dola, Opatha	6.266879°N, 80.40226°E	Nilwala	62	**PQ842771**	**PQ848171**	NA	**PQ848244**	50
DZ4477	*Devario pathirana*	Ugudu dola, Opatha	6.266879°N, 80.40226°E	Nilwala	62	**PQ842772**	**PQ848172**	**PQ848327**	**PQ848245**	50
DZ4524	*Devario malabaricus*	Keppetigala	7.435449°N, 80.54643°E	Deduru	446	**PQ842773**	**PQ848173**	**PQ848328**	**PQ848246**	51
DZ4530	*Devario malabaricus*	Molagoda, Kegalle	7.256279°N, 80.39134°E	MaaOya	108	**PQ842774**	**PQ848174**	**PQ848329**	**PQ848247**	52
DZ4531	*Devario malabaricus*	Molagoda, Kegalle	7.256279°N, 80.39134°E	MaaOya	108	**PQ842775**	**PQ848175**	**PQ848330**	NA	52
DZ4541	*Devario malabaricus*	Dambulu Oya, Dambulla	7.808347°N, 80.65845°E	KalaOya	189	**PQ842776**	**PQ848176**	**PQ848331**	NA	53
DZ4555	*Devario malabaricus*	Diyakobala road, Kotagama	7.131992°N, 81.18182°E	GalOya	289	**PQ842777**	**PQ848177**	**PQ848332**	NA	54
DZ4556	*Devario malabaricus*	Diyakobala road, Kotagama	7.131992°N, 81.18182°E	GalOya	289	**PQ842778**	**PQ848178**	**PQ848333**	NA	54
DZ4605	*Devario malabaricus*	Yudaganawa, Buttala	6.759839°N, 81.22552°E	Menik	163	**PQ842779**	**PQ848179**	**PQ848334**	NA	55
DZ4725	*Devario micronema*	Indikada-dola, Weralugahamula, Rakwana	6.487614°N, 80.59929°E	Kalu	311	**PQ842780**	**PQ848180**	**PQ848335**	NA	56
DZ4726	*Devario micronema*	Indikada-dola, Weralugahamula, Rakwana	6.487614°N, 80.59929°E	Kalu	311	**PQ842781**	**PQ848181**	**PQ848336**	**PQ848248**	56

DZ	Genus\Species	Location	GPS Coordinates	River basin	Elevation (m asl)	*cox1*	*cytb*	*rag1*	*irbp*	Index
DZ4727	*Devario micronema*	Indikada-dola, Weralugahamula, Rakwana	6.487614°N, 80.59929°E	Kalu	311	**PQ842782**	**PQ848182**	**PQ848337**	**PQ848249**	56
DZ4758	*Devario micronema*	Induruwa FR, Gilimale	6.763923°N, 80.42889°E	Kalu	97	**PQ842783**	**PQ848183**	**PQ848338**	NA	57
DZ4759	*Devario micronema*	Induruwa FR, Gilimale	6.763923°N, 80.42889°E	Kalu	97	**PQ842784**	**PQ848184**	**PQ848339**	**PQ848250**	57
DZ4760	*Devario micronema*	Induruwa FR, Gilimale	6.763923°N, 80.42889°E	Kalu	97	**PQ842785**	**PQ848185**	**PQ848340**	**PQ848251**	57
DZ4775	*Devario micronema*	Runakanda FR, Mathugama	6.454762°N, 80.32631°E	Kalu	113	**PQ842786**	**PQ848186**	**PQ848341**	**PQ848252**	58
DZ4776	*Devario micronema*	Runakanda FR, Mathugama	6.454762°N, 80.32631°E	Kalu	113	**PQ842787**	**PQ848187**	**PQ848342**	NA	58
DZ4777	*Devario micronema*	Runakanda FR, Mathugama	6.454762°N, 80.32631°E	Kalu	113	**PQ842788**	**PQ848188**	**PQ848343**	**PQ848253**	58
DZ4794	*Devario malabaricus*	Maha Elagamuwa	7.989792°N, 80.61841°E	KalaOya	150	**PQ842789**	**PQ848189**	**PQ848344**	**PQ848254**	59
DZ4810	*Devario* cf. *malabaricus*	Mahawala, Udadumbara	7.304308°N, 80.94199°E	Mahaweli	682	**PQ842790**	**PQ848190**	**PQ848345**	**PQ848255**	60
DZ4811	*Devario* cf. *malabaricus*	Mahawala, Udadumbara	7.304308°N, 80.94199°E	Mahaweli	682	**PQ842791**	**PQ848191**	**PQ848346**	**PQ848256**	60
DZ4822	*Devario malabaricus* × *micronema* hybrid	Dellawa	6.37522°N, 80.41204°E	Gin	125	**PQ842792**	**PQ848192**	**PQ848347**	NA	61
DZ4833	*Devario malabaricus* × *micronema* hybrid	Dellawa	6.37522°N, 80.41204°E	Gin	125	**PQ842793**	**PQ848193**	**PQ848348**	**PQ848257**	61
DZ4849	*Devario malabaricus*	Nelum Wewa, Eluwankulama	8.279778°N, 79.87542°E	KalaOya	7	**PQ842794**	**PQ848194**	**PQ848349**	NA	62
DZ4876	*Devario malabaricus*	Rajanganaya	8.141471°N, 80.22109°E	KalaOya	57	**PQ842795**	**PQ848195**	**PQ848350**	NA	63
DZ4976	*Devario malabaricus* × *micronema* hybrid	Near Hiniduma Rest House, Hiniduma	6.303326°N, 80.31934°E	Gin	27	**PQ842796**	**PQ848196**	**PQ848351**	NA	64
DZ4977	*Devario malabaricus* × *micronema* hybrid	Near Hiniduma Rest House, Hiniduma	6.303326°N, 80.31934°E	Gin	27	**PQ842797**	**PQ848197**	**PQ848352**	NA	64
DZ4978	*Devario malabaricus* × *micronema* hybrid	Near Hiniduma Rest House, Hiniduma	6.303326°N, 80.31934°E	Gin	27	**PQ842798**	**PQ848198**	**PQ848353**	**PQ848258**	64
DZ5075	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842799**	**PQ848199**	**PQ848354**	**PQ848259**	65
DZ5076	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842800**	**PQ848200**	**PQ848355**	**PQ848260**	65
DZ5077	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842801**	**PQ848201**	**PQ848356**	**PQ848261**	65
DZ5078	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842802**	**PQ848202**	**PQ848357**	**PQ848262**	65
DZ5079	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842803**	**PQ848203**	**PQ848358**	**PQ848263**	65
DZ5080	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842804**	**PQ848204**	**PQ848359**	**PQ848264**	65
2025.04.01. NH (DZ5081)	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842805**	**PQ848205**	NA	**PQ848265**	65
2025.04.02. NH (DZ5082)	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842806**	**PQ848206**	**PQ848360**	**PQ848266**	65
DZ5083	*Devario altus* sp. nov	Bopathalawa, MenikPalama Farm	6.815751°N, 80.70651°E	Mahaweli	1580	**PQ842807**	**PQ848207**	**PQ848361**	**PQ848267**	65
DZ5134	*Devario malabaricus*	Thulawelliya, Kanadara Oya	8.509139°N, 80.42289°E	MalwathuOya	66	**PQ842808**	**PQ848208**	**PQ848362**	NA	39
DZ5135	*Devario malabaricus*	Thulawelliya, Kanadara Oya	8.509139°N, 80.42289°E	MalwathuOya	66	**PQ842809**	**PQ848209**	**PQ848363**	**PQ848268**	39

The methods of DNA extraction, PCR amplification, and PCR product purification for *cytb*, *cox1*, *rag1*, and *irbp* followed those described by Sudasinghe et al. (2023a). ChromasPro v1.34 (Technelysium Pty Ltd, Australia) and MEGA v7.0 (Kumar et al. 2016) were employed to verify the newly generated sequences and create the consensus sequences for the 5' and 3' strands, respectively.

As shown by Sudasinghe et al. (2020a), the five species of Sri Lankan *Devario* are the result of a monophyletic insular diversification. The present study aims to elucidate the fine-scale phylogenetic and phylogeographic patterns within this diversification. Therefore, as outgroups, we selected only species of *Devario* for which at least three of the four molecular markers used in this study were already available in GenBank: *D. anomalus* (CTOL03308), *D. coxi* (CTOL03311), *D. regina* (CTOL02866), and *D. xyrops* (NRM 41674), all of which occur in or east of the Ganges River basin and are hence absent from the peninsula of India.

The sequences for *cytb* (1092 bp), *cox1* (642 bp), *rag1* (1484 bp), and *irbp* (727 bp) were aligned using MUSCLE (Edgar 2004) in MEGA. Each alignment was reviewed and translated to confirm the absence of frameshift mutations and premature stop codons. Data concatenation and sequence format conversion were performed using FASconCAT-G (Kück and Longo 2014).

Phylogenetic analysis

Phylogenetic inference using the Maximum Likelihood (ML) method using IQ-TREE 2 (Minh et al. 2020) was conducted for each individual gene alignment of *cytb* (139 individuals), *cox1* (140 individuals), *rag1* (111 individuals), and *irbp* (60 individuals). The concatenated mitochondrial dataset (1734 bp, 140 individuals) is referred to as the 'mtDNA dataset', while the concatenated nuclear dataset (2211 bp, 114 individuals) is referred to as the 'nuDNA dataset'. The optimal nucleotide substitution model and dataset partitions were determined with ModelFinder (Kalyaanamoorthy et al. 2017), starting with each codon position as a separate subset. Node support in the ML tree was assessed through ultrafast bootstrapping (BS) with 1000 iterations (Hoang et al. 2018). IQ-TREE 2 analyses were executed on UBELIX (http://www.id.unibe.ch/hpc), the HPC cluster at the University of Bern, Switzerland. The resulting ML trees were visualized using Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). Additionally, to visualize the non-tree-like signal, a phylogenetic network was constructed using the Neighbor-Net algorithm based on uncorrected-P distances with SplitsTree v.4.18.2 (Huson and Bryant 2006) using the concatenated mitochondrial and nuclear dataset (3945 bp, 140 individuals), referred to as the 'total dataset'.

To understand phylogeographic patterns, haplotype networks for each mitochondrial marker were reconstructed by Median Joining Network (Bandelt et al. 1999) in PopArt (Leigh and Bryant 2015). Uncorrected pairwise genetic distances in *cox1* were calculated using MEGA. For the two most widespread species, *D. malabaricus* and *D. micronema*, each with a sample size > 25, demographic histories were explored for the *cytb* and *cox1* mitochondrial markers by computing the number of haplotypes (h), polymorphic sites (S), parsimony-informative sites (P), nucleotide diversities (π), haplotype diversities (Hd), and Tajima's D (Tajima 1989) and Fu and Li's F (Fu and Li 1993) neutrality tests using DNAsp v.6 (Rozas et al. 2017).

Morphology

External morphology was examined in a total of 363 specimens representative of all the Sri Lankan species of *Devario*, based on the collection of the Wildlife Heritage Trust of Sri Lanka (WHT, now in the National Museum of Sri Lanka, Colombo: NMSL) and the Evolutionary Ecology and Systematics Lab, Department of Molecular Biology and Biotechnology, University of Peradeniya, Peradeniya (DZ) (Suppl. material 1). In addition, the type specimens of *D. micronema* and *D. monticola* (=*D. lineolatus*) at the Naturalis Biodiversity Center, Leiden (RMNH) were also examined (Sudasinghe et al. 2025). Measurements were taken point-to-point, to the nearest 0.1 mm, on the left side of specimens using digital calipers. Methods for counts and measurements follow Sudasinghe (2024).

Statistical analyses were conducted using R Statistical Software 4.0.0 (R Core Team 2020). The dataset, consists of 33 traits, 21 of which are linear measurements made point-to-point: Standard length (SL), Predorsal length (PDL), Postdorsal length (PoDL), Preanal length (PAL), Prepelvic length (PPL), Caudal peduncle length (CPL), Caudal peduncle depth (CPD), Body depth (BD), Dorsal fin height (DFH), Dorsal-fin base length (DFBL), Anal fin height (AFH), Anal-fin base length (AFBL), Pelvic fin height (PFH), Pectoral fin height (PeFH), Head length (HL), Head depth (HD), Snout length (SNL), Eye diameter (ED), Inter-orbital width (IOW), Rostral-barbel length (RosB), Maxillary barbel length (MaxB); and 12 of which are meristic counts: pored lateral-line scales (LL), predorsal scales (PreDS), prepelvic scales (PrePS), circumpeduncular scales (CS), scales between lateral line and dorsal-fin origin (LLD), scales between lateral line and anal-fin origin (LLA), scales between lateral line and pelvic-fin origin (LLP), branched dorsal-fin rays (DF), branched anal-fin rays (AF), branched pelvic-fin rays (PF), branched caudal-fin rays (CF) and branched pectoral-fin rays (PeF). All the traits were first log-transformed prior to analysis. Using standard length as the size axis, the other 32 traits were plotted against SL to check for positive correlation and to identify any potential outliers. All linear measurements showed a positive correlation with SL, and no outliers were detected. Among the meristic traits, CF did not show any variation and was excluded from further statistical analyses, thus resulting in a total of 32 variable traits.

Principal Component Analyses (PCA) were performed to visualize and summarize the multivariate morphometric data. Morphometric analyses were carried out for three different datasets. The first, referred to as the 'species dataset' (n=279), included only specimens confidently identified to each species. This was used to determine whether the various species groups of Sri Lankan *Devario* formed distinct clusters in the morpho-space. The second, the 'malabaricus-altus dataset' (n=199), included *D. malabaricus*, *D. altus* sp. nov., and *D.* cf. *malabaricus*. It was used to assess the morphological similarity of *D.* cf. *malabaricus* to *D. malabaricus* or *D. altus* sp. nov. The third, the 'malabaricus-micronema-hybrid dataset' (n=255), comprised *D. malabaricus*, *D. micronema*, and putative hybrids of *D. malabaricus* and *D. micronema*, to assess their morphological similarity to either *D. malabaricus* or *D. micronema*.

All three datasets were size-corrected using the methods of Berner (2011) and Roesti et al. (2023) by performing a pooled within-group regression, with species identity provided as the 'group' during size correction. In this method, an ANCOVA is conducted for each species and trait, using the log-transformed 'trait' as the response variable, 'species' as a factor, and log-transformed 'size' as a covariate. Then, to obtain the size-corrected individual trait values, the residuals are added to the predicted trait value for each species at the mean size across all individuals. The PCAs were then conducted using a correlation matrix to visualize and summarize the multivariate morphometric and meristic data in multiple dimensions. The R package FactoMineR (version 1.34) (Lê et al. 2008) was used to conduct the PCA. To test whether the principal components of the PCA were significantly different among the species, an ANCOVA was conducted using the function lm () with the syntax R: lm (PC ~ species). The function lm () is available as part of the base R. Type III 'sums of squares' available in the R-package 'car' (Fox and Weisberg 2018) was used to calculate the F-statistics.

A one-way ANOVA using the aov () function in R was conducted to evaluate body size differences among the species of Sri Lankan *Devario*. Standard length (SL) served as a proxy for body size. Post-hoc comparisons were performed using the Tukey HSD method in base R to identify specific size differences between species. Additionally, to determine which traits differed significantly between species, an ANCOVA was performed using the lm function in R. Each log-transformed trait was treated as the response variable, with SL included as a covariate in the model: lm (trait ~ species+SL).

The presence or absence of the infraorbital process, a laterally directed dorsal extension of the posterior edge of infraorbital 1 (Kottelat 2020a), is widely considered a useful trait in taxonomy of *Devario* (Conway et al. 2009; Fang and Kullander 2009; Kullander et al. 2017; Kottelat 2020a; Kullander and Norén 2022). To detect this character state, all specimens were examined under a stereo microscope. A Fisher's Exact Test was carried out to assess whether the presence/absence of this trait is significantly different among the various species.

Most species of *Devario* undergo gradual ontogenetic changes in color pattern, with adults typically displaying species-specific patterns of stripes, blotches, and bars on the flank (Kullander and Norén 2022). These adult color patterns are widely used in the taxonomy of *Devario* (Fang 1997a, b; Fang and Kottelat 1999; Conway et al. 2009; Fang and Kullander 2009; Kullander 2017; Kullander et al. 2017; Kottelat 2020a, b; Kullander and Norén 2022). In this study, the color patterns of all specimens were examined following the terminology of Fang (1998), as modified by Kullander (2015) (Fig. 2). For a subset (n=145) of the 'malabaricus-altus dataset' for which photographs of the specimens were available, the length of the I+1 interstripe ({I+1}) was measured using ImageJ (Schneider et al. 2012) and the proportion {I+1}/SL calculated. The statistical significance of the variation of {I+1}/SL was tested using a one-way ANOVA and post-hoc comparisons were performed using the Tukey HSD method. Additionally, for this dataset, we examined whether or not the P-stripe was bifurcated anteriorly and tested whether or not this was statistically significant by conducting a Fisher's Exact Test.

Results

Molecular analysis: mitochondrial phylogeny

In the mitochondrial DNA (mtDNA) phylogeny (Fig. 3A), *Devario micronema*, *D. pathirana*, and *D. memorialis* form a well-supported monophyletic clade, with *D. memorialis* being recovered as the sister group of the clade comprising *D. micronema* and *D. pathirana* (bootstrap support value [BS]=95). All three species are confined to the island's southwestern wet zone. All the samples (n=9) from Bopathalawa (1580 m asl), which were phenotypically identified as *D. altus* sp. nov., form a well-supported monophyletic clade. *Devario altus* sp. nov. was recovered as having a sister group relationship to *D. malabaricus* (BS=100). The clade comprising *D. malabaricus* and *D. altus* sp. nov. was recovered as a well-supported sister group to the clade that includes *D. micronema*, *D. pathirana*, and *D. memorialis* (BS=100). Specimens identified as *D.* cf. *malabaricus*, exhibiting a color pattern similar to *D. altus* sp. nov., are found to be clustered within both the *D. malabaricus* and *D. altus* sp. nov. clades.

Molecular analysis: nuclear phylogeny

Contrasting with the mtDNA phylogeny, the nuDNA phylogeny (Fig. 3B) does not recover the monophyly of the five species as clearly. *Devario malabaricus* is split into two paraphyletic clades. One of these, which contains only two samples of *D. malabaricus* and two samples of *D.* cf. *malabaricus*, is identified as the sister group of

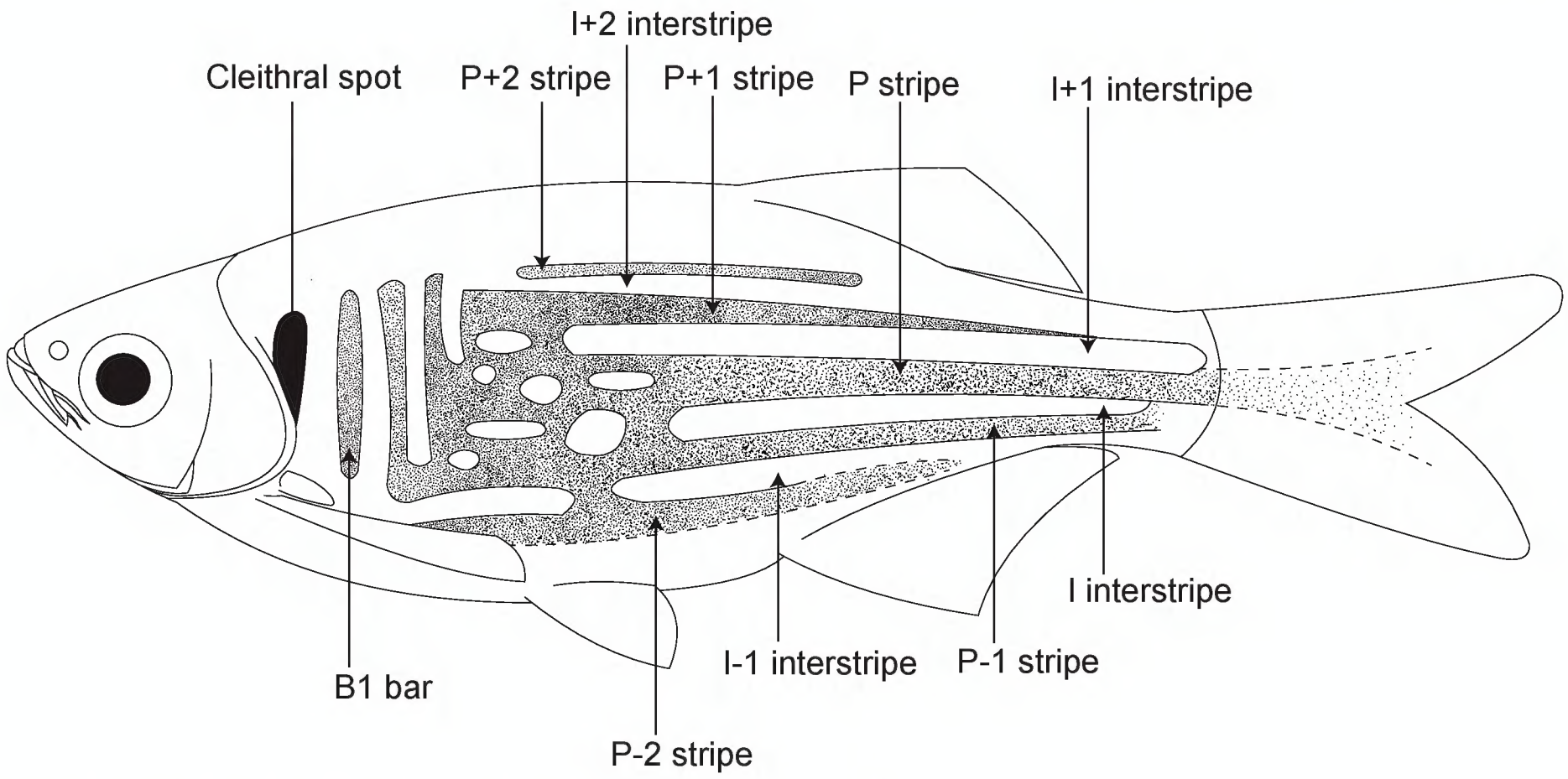

Figure 2. Color-pattern terminology used for *Devario* in the present study.

the remaining species of *Devario* in Sri Lanka (BS=100). The other samples of *D. malabaricus*, along with *D.* cf. *malabaricus*, *D. memorialis*, and *D. altus* sp. nov., are clustered together. Furthermore, *Devario pathirana* is nested within *D. micronema*.

Comparison of mtDNA and nuDNA phylogenies

The comparison of mtDNA and nuDNA phylogenies reveals mitonuclear discordance between *D. pathirana*, *D. micronema*, and *D. malabaricus*. Sample DZ3337 from Suriyakanda in the Walawe basin clusters with *D. pathirana* in the mtDNA phylogeny but aligns with *D. malabaricus* in the nuDNA phylogeny (Fig. 3). In contrast, sample DZ3336 from the same locality clusters with *D. malabaricus* in the mtDNA phylogeny but with *D. pathirana* in the nuDNA phylogeny (Fig. 3).

Meanwhile, sample DZ4978 from Hiniduma in the Gin basin clusters with *D. malabaricus* in both the mtDNA and the nuDNA phylogenies. Two other samples from the same site, DZ4976 and DZ4977, however, cluster with *D. micronema* in the mtDNA phylogeny and with *D. malabaricus* in the nuDNA phylogeny (Fig. 3). Additionally, sample DZ3406 from Andawaldola in the Kalu basin clusters with *D. micronema* in the mtDNA phylogeny but with *D. malabaricus* in the nuDNA phylogeny. However, another sample from this locality, DZ3405, clusters with *D. malabaricus* in both the mtDNA and the nuDNA phylogenies (Fig. 3).

Although sample DZ4833 from Dellawa in the Gin basin clusters with *D. malabaricus* in both the mtDNA and the nuDNA phylogenies, another sample from the same locality, DZ4822, clusters with *D. malabaricus* in the mtDNA phylogeny but with *D. micronema* in the nuDNA phylogeny (Fig. 3).

Phylogenetic network

The phylogenetic network constructed using the total dataset supports the monophyly of each of the five Sri Lankan species of *Devario* (Fig. 4). The clades of *D. malabaricus* and *D. altus* sp. nov. are phylogenetically closer to each other. In contrast, the clades of *D. micronema*, *D. pathirana*, and *D. memorialis* are more distantly related to *D. malabaricus* and *D. altus* sp. nov.

However, the network indicates some uncertainties in the connections between different species, likely due to hybridization events and/or incomplete lineage sorting. Of the five species, populations of *D. micronema* show increased reticulation compared to the rest (Fig. 4).

Genetic distances

Fig. 5 illustrates the uncorrected pairwise genetic distances for the *cox1* gene across the various species of *Devario* in Sri Lanka. The analysis reveals that each of the five species has an intraspecific genetic divergence of less than 1%. In contrast, interspecific genetic divergence between all species pairs is greater than 1%. The smallest divergence, 1.2%, is observed between *D. pathirana* and *D. micronema*, and between *D. altus* sp. nov. and *D. malabaricus*. At the other end of the spectrum, the greatest interspecific genetic divergence occurs between *D. malabaricus* and *D. memorialis*: 4.8%.

Genetic diversity and phylogeography

For the *cox1* gene, *Devario malabaricus* (n=50) revealed a nucleotide diversity (π) of 0.00157 and a haplotype diversity (Hd) of 0.694. Tajima's D value was -1.75803,

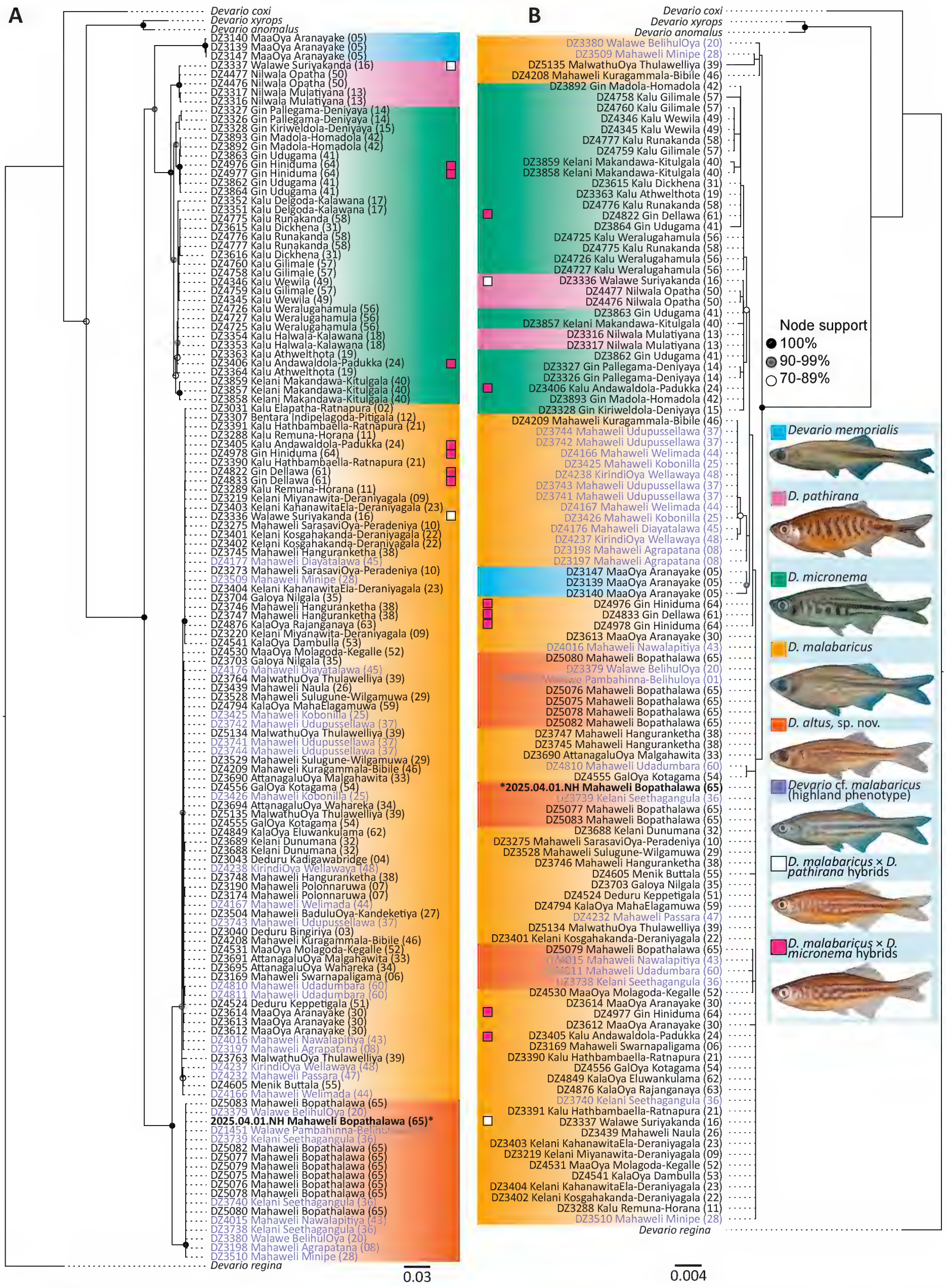

Figure 3. Molecular phylogenetic relationships of Sri Lankan *Devario* based on maximum likelihood inference of **A.** The concatenated mitochondrial dataset (1734 bp, 140 individuals); **B.** The concatenated nuclear dataset (2211 bp, 114 individuals). Node support represents ultrafast bootstrapping for 1000 iterations. Node support below 70 is not labeled. Scale bar represents number of changes per site. Numbers in parentheses indicate the sampling localities listed in Table 1. The hologenetype of *Devario altus*, sp. nov. is indicated in bold and with an asterisk.

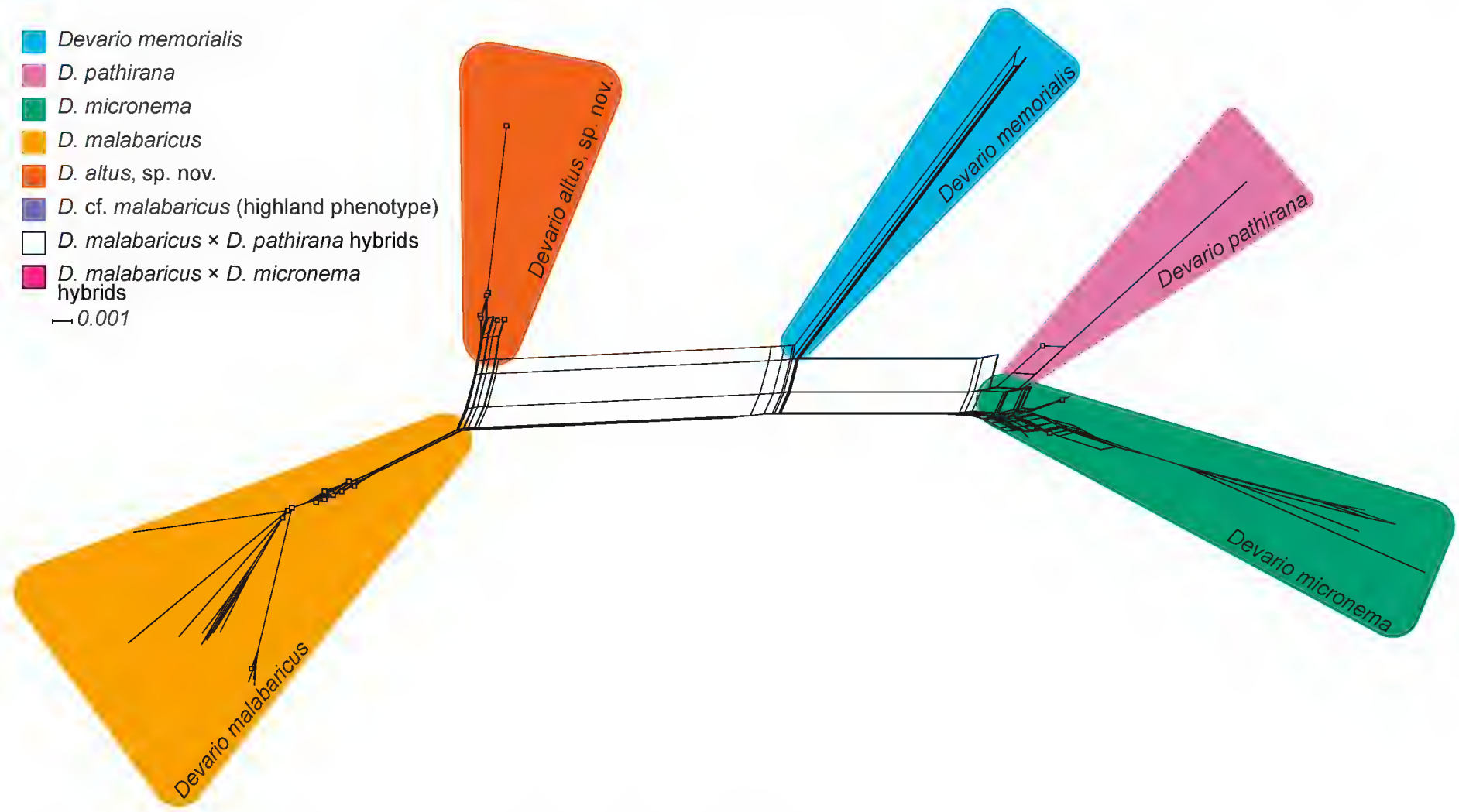

Figure 4. Phylogenetic network based on the Neighbor-Net algorithm based on uncorrected-P distances with SplitsTree for the concatenated mitochondrial and nuclear dataset (3945 bp, 136 individuals).

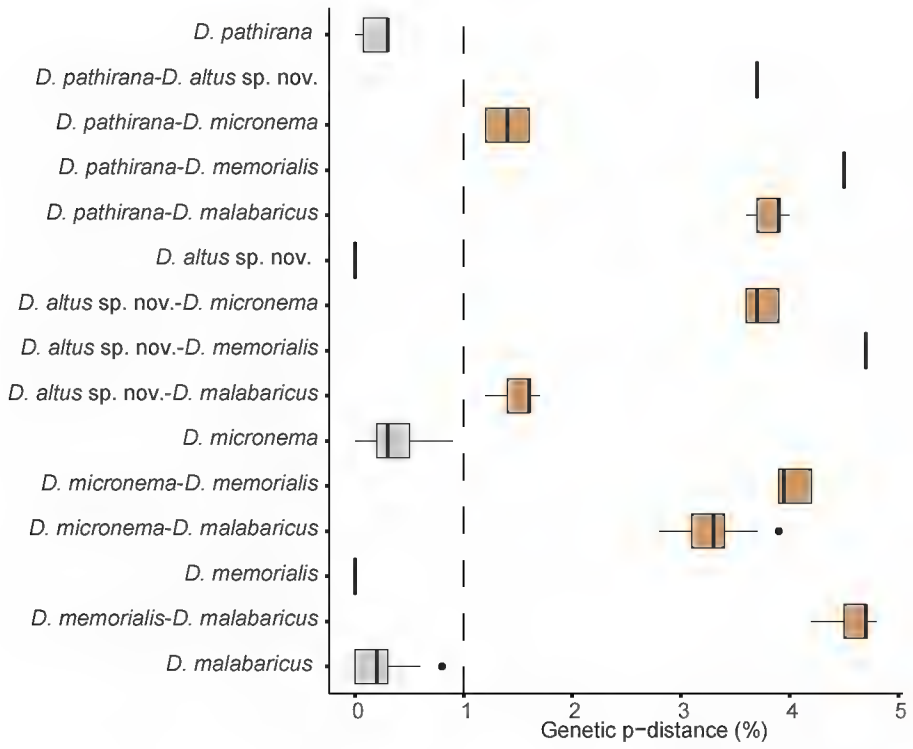

Figure 5. Intraspecific (gray) and interspecific (brown) genetic variation for mitochondrial *cox1* molecular marker in Sri Lankan *Devario*. Dashed line represents the genetic gap between intra- and inter-specific genetic variation.

which was not statistically significant ($0.10 > P > 0.05$), indicating no significant deviation from neutrality. Fu and Li's F* test statistic for this species was -2.72446, with a significance level of $P < 0.05$. The analysis identified 11 segregating sites, of which 4 were parsimony-informative.

In contrast, *D. micronema* (n=30) exhibited a higher nucleotide diversity (π) of 0.00350 and haplotype diversity (Hd) of 0.802. Tajima's D value was -0.70032, which was not statistically significant ($P > 0.10$), while Fu and Li's F* test statistic was 0.10025, also not significant ($P > 0.10$). This species had 11 segregating sites, 9 of which were parsimony informative.

For the *cytb* gene, *D. malabaricus* (n=50) revealed a nucleotide diversity (π) of 0.00348 and a haplotype diversity (Hd) of 0.798. Tajima's D value was -1.57609, which was not statistically significant ($0.10 > P > 0.05$). Fu and Li's F* test statistic was -3.53030, with a significance level of $P < 0.02$. Thirty-one segregating sites were identified, of which ten were parsimony-informative.

Devario micronema (n=29), on the other hand, showed a higher nucleotide diversity (π) of 0.00850 and haplotype diversity (Hd) of 0.892. Tajima's D value was -0.23250, which was not statistically significant ($P > 0.10$), while Fu and Li's F* test statistic was 0.30904, also not significant ($P > 0.10$). This species had 37 segregating sites, of which 30 were parsimony-informative.

Despite *D. malabaricus* being geographically more widespread than *D. micronema* (Figs 6, 7), *D. micronema* exhibits, in both the *cox1* and *cytb* mitochondrial markers, greater haplotype and nucleotide diversity than *D. malabaricus*.

Analysis of the *cytb* and *cox1* haplotype networks (Fig. 6) revealed that *D. micronema* exhibits a strong phylogeographic structure, with distinct haplotypes that are not shared between adjacent basins. This indicates strong genetic differentiation across its geographical range. Such structure is not evident in *D. malabaricus*, despite it being geographically more widespread, suggesting a higher degree of gene flow among its populations (Fig. 7).

Haplotype H1 in Fig. 7B, C is represented in all the samples of *D. altus* sp. nov. from Bopathalawa (1580 m asl). Several samples of *D.* cf. *malabaricus* from the Mahaweli, Kelani, Walawe, and Kirindi basins, the headwaters of which converge in this region, also share this haplotype in both *cox1* and *cytb* (Fig. 7B, C).

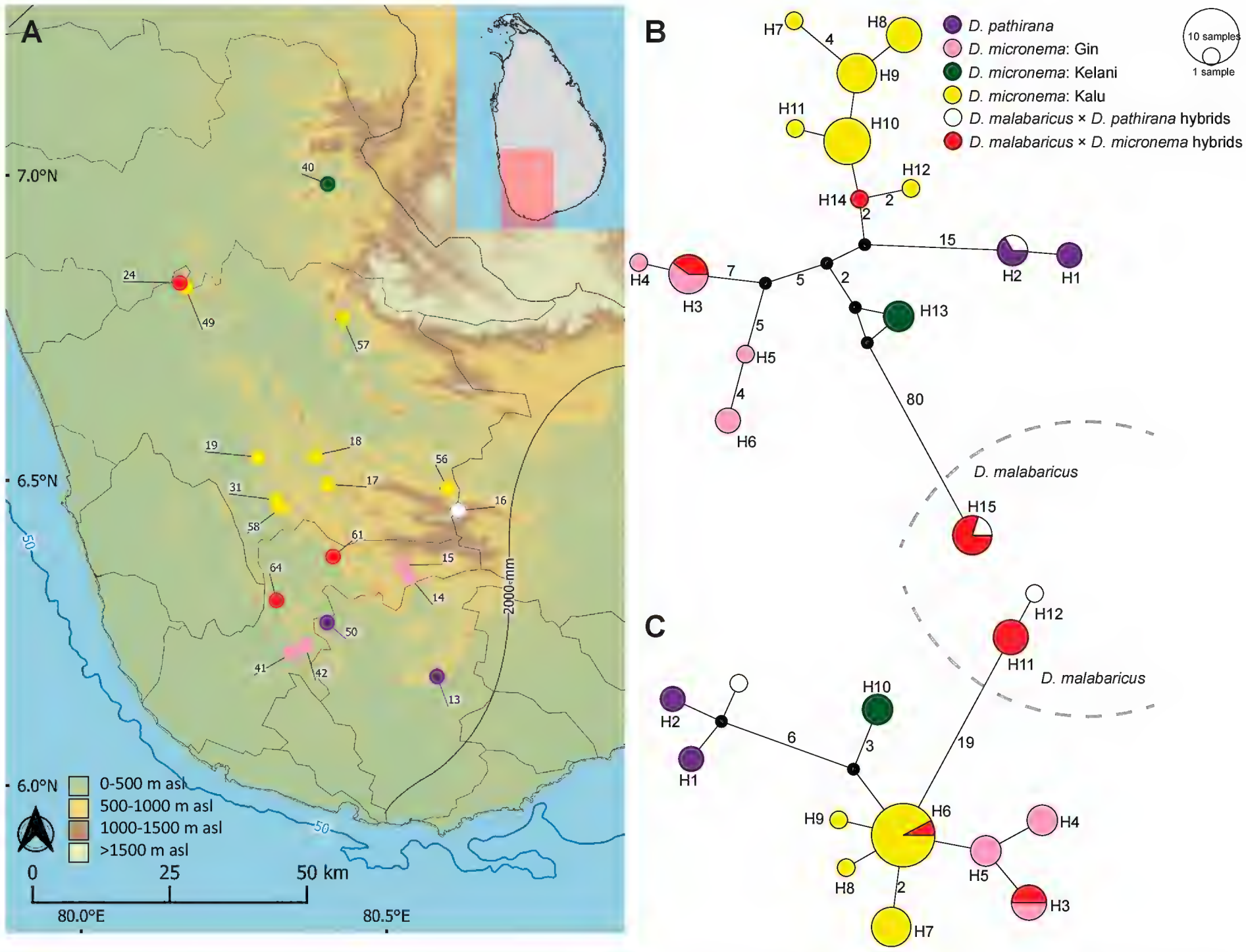

Figure 6. A. Geographical origin of samples of *Devario micronema*, *D. malabaricus* × *D. pathirana* hybrids, and *D. malabaricus* × *D. micronema* hybrids. Numbers on the map represent the sampling localities listed in Table 1. Median-joining haplotype networks based on the analysis of **B.** A 1092 bp fragment of the *cytb* gene; **C.** A 642 bp fragment of the *cox1* gene. The number of mutational steps >1 is shown. The areas of the circles are proportional to the number of individuals sharing a given haplotype. Black circles indicate hypothetical nodes. Legend colors correspond to river basins or to the phenotype. Major river-basin boundaries are marked with narrow black lines.

Morphological analysis: the 'species dataset'

Suppl. material 2 provides the morphometric and meristic data relating to the material examined. The one-way ANOVA on the 'species dataset' reveals significant differences in log-transformed standard length ($F = 12.14$, $p = 4.22e\text{-}09$; Fig. 8A). The Tukey HSD post hoc test shows significant size differences between the following species pairs: *D. micronema* and *D. malabaricus* ($p < 0.001$), *D. altus* sp. nov. and *D. malabaricus* ($p = 0.004$), *D. pathirana* and *D. micronema* ($p = 0.025$), and *D. pathirana* and *D. altus* sp. nov. ($p = 0.037$).

In the PCA analysis of log-transformed, size-corrected morphometric and meristic species dataset, PC1 and PC2 collectively accounted for 35.9% of the total variance (Suppl. material 3). The PC1 vs PC2 plot revealed distinct clusters for the five species in morphological space, indicating interspecific divergences in morphological traits (Fig. 9A). The species differed significantly along the major axis of morphological variation in PC1, explaining 26.1% of the total variation (Fig. 9A: 'species' [$F= 155.56$, $p<0.0001$]); and PC2, explaining 9.8% of the total variation (Fig. 9A; 'species' [$F= 68.035$, $p<0.0001$]).

Devario altus sp. nov. and *D. memorialis* are well separated in morpho-space, while *D. malabaricus*, *D. micronema*, and *D. pathirana* are clustered. Along the PC1 axis, the heavily loaded positive traits were larger on average in *D. malabaricus*, *D. micronema*, and *D. pathirana* compared to *D. memorialis* and *D. altus* sp. nov. (Fig. 9A). The most heavily loaded traits on PC1 are body depth, prepelvic length, dorsal-fin base length, inter-orbital width, and branched dorsal-fin rays (Suppl. material 3). Along the PC2 axis, the heavily loaded positive traits were larger on average in *D. altus* sp. nov. compared to the remaining species (Fig. 9A). The most heavily loaded traits on PC2 are caudal peduncle length, anal-fin base length, scales between lateral line and anal-fin origin, and branched anal-fin rays (Suppl. material 3).

Among the 31 morphometric traits analyzed in the ANCOVA, interspecific differences explain significant degrees of morphological variation (Suppl. material 2). Specifically, all traits except AFH and MaxB show an

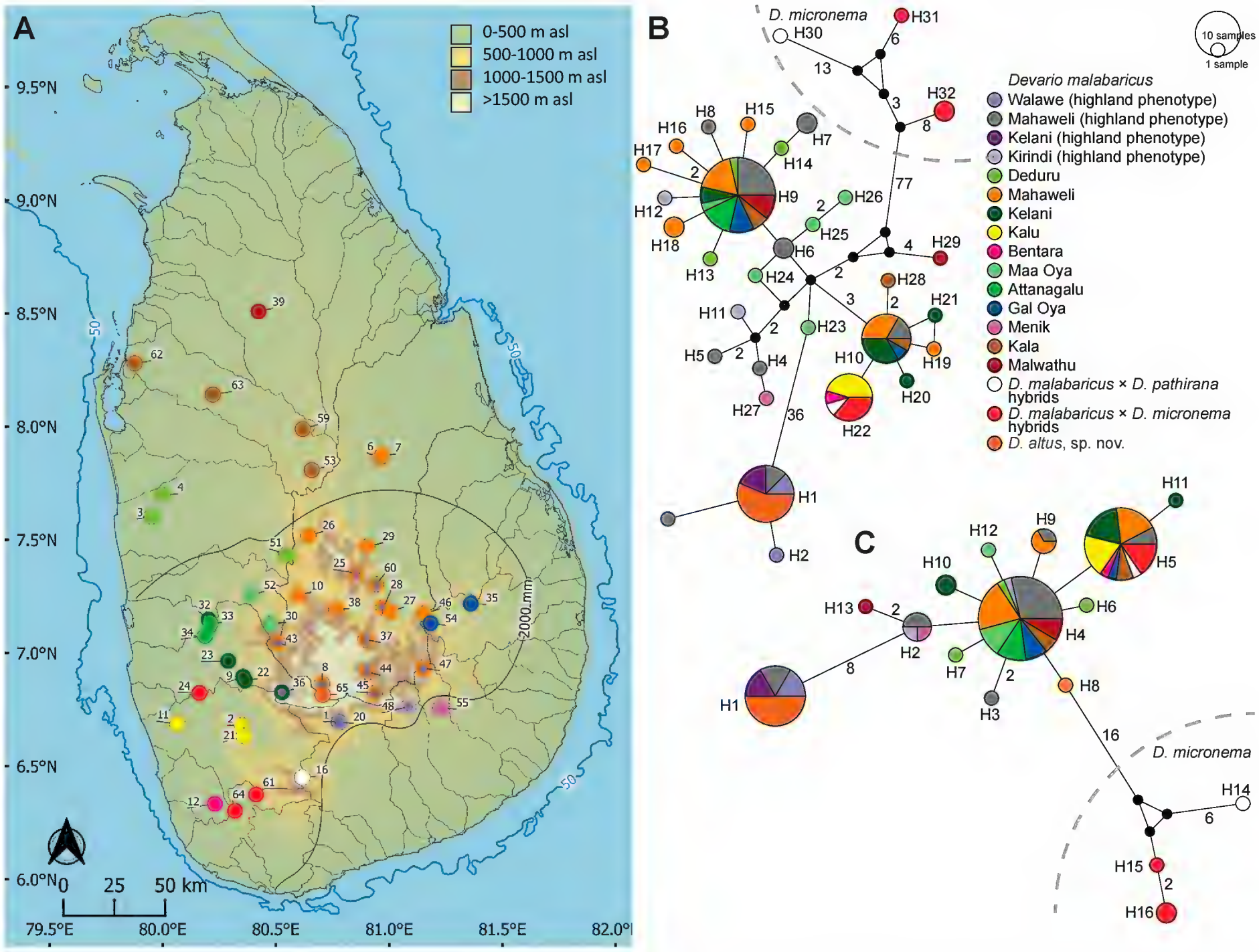

Figure 7. A. Geographical origin of samples of *Devario malabaricus*, *D. altus* sp. nov., *D.* cf. *malabaricus* (highland phenotype), *D. malabaricus* × *D. pathirana* hybrids, and *D. malabaricus* × *D. micronema* hybrids. Points with an inner circle represent populations representing *D.* cf. *malabaricus* (highland phenotype). Numbers on the map represent the sampling localities listed in Table 1. Median-joining haplotype networks based on the analysis of **B.** A 1092 bp fragment of the *cytb* gene; **C.** A 642 bp fragment of the *cox1* gene. The number of mutational steps >1 is shown. The areas of the circles are proportional to the number of individuals sharing a given haplotype. Black circles indicate hypothetical nodes. Legend colors correspond to river basins or to the phenotype. Major river-basin boundaries are marked with narrow black lines.

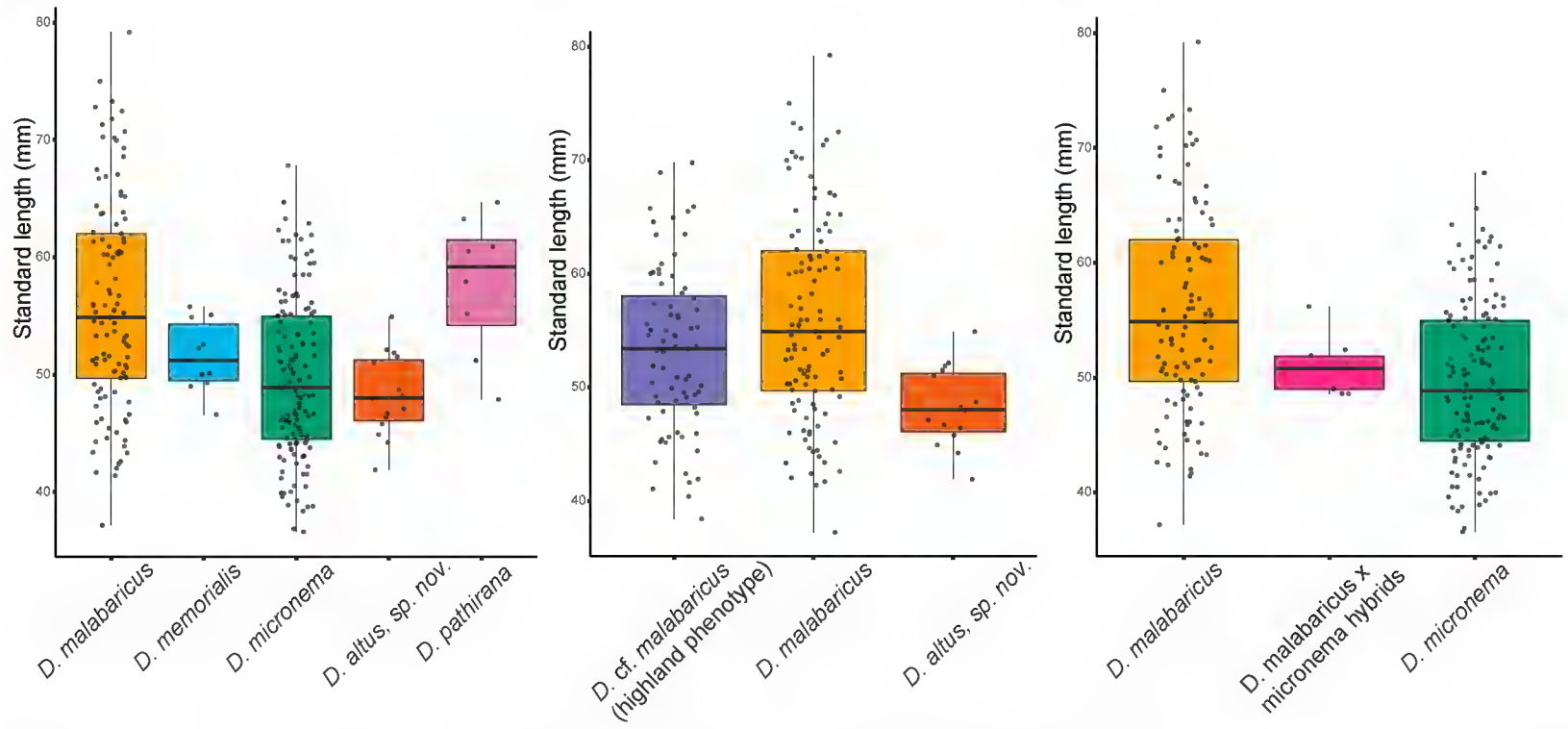

Figure 8. Variation of standard length in **A.** The 'species dataset' (n=279); **B.** The 'malabaricus-altus dataset' (n=199); **C.** The 'malabaricus-micronema-hybrid dataset' (n=255).

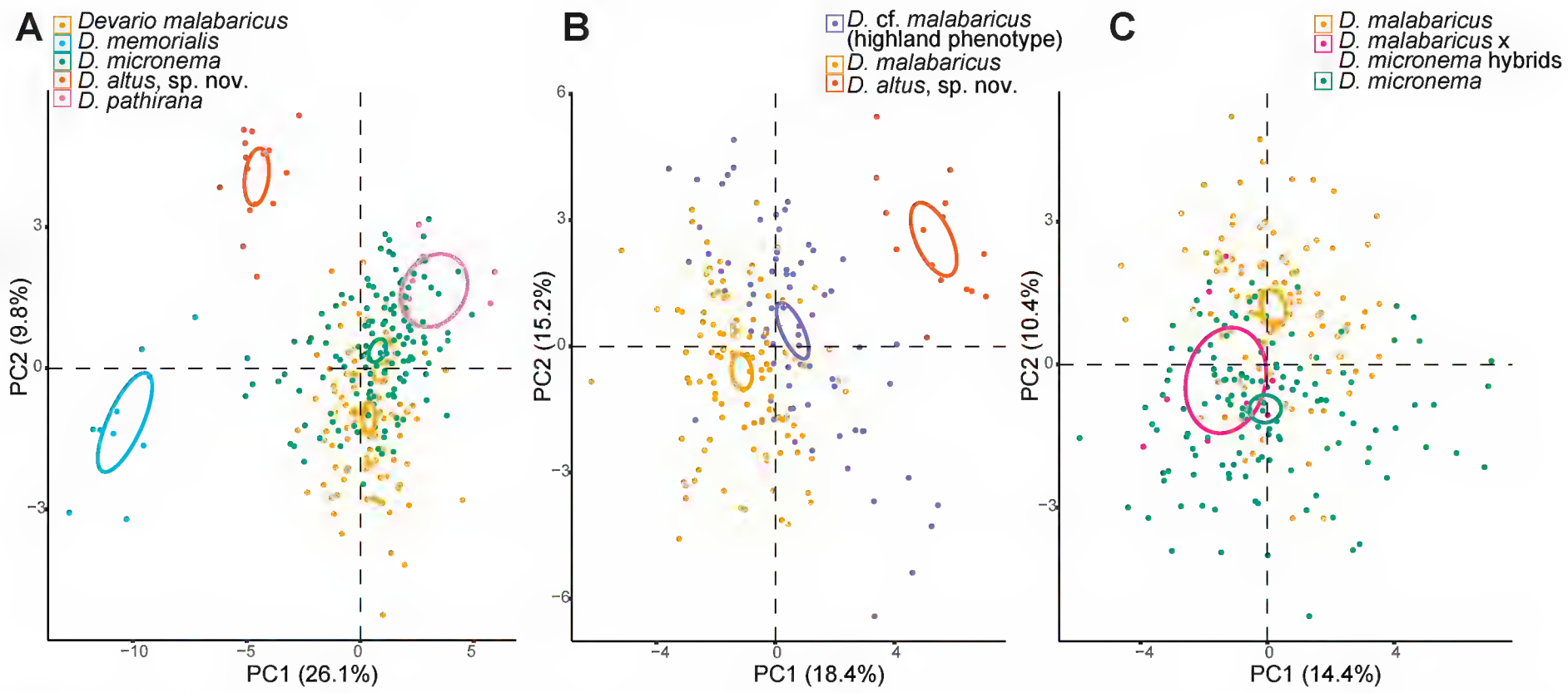

Figure 9. PCA biplot of the first two principal components of morphological variation in size-corrected measurements and meristics of **A.** The 'species dataset' (n=279); **B.** The 'malabaricus-altus dataset' (n=199); **C.** The 'malabaricus-micronema-hybrid dataset' (n=255). Ellipses represent the confidence interval around group mean points.

independent, statistically significant effect of 'species' in the ANCOVA (Suppl. material 2).

The frequency of the presence or absence of the infraorbital process in the five species is shown in Fig. 10A. The infraorbital process is present in all 8 specimens of *D. pathirana* examined and absent in all 10 specimens of *D. memorialis* examined. In *D. altus* sp. nov., the process is present in 11 specimens and absent in 4, while in *D. micronema* it is present in 132 specimens and absent in only 5. In contrast, in *D. malabaricus*, the process is absent in 103 and present in only 6 specimens. Based on Fisher's Exact Test, the differences in the presence or absence of the infraorbital process is statistically significant between the species ($p < 2.2e\text{-}16$).

Morphological analysis: the 'malabaricus-altus dataset'

Suppl. material 4 provides morphometric and meristic data relating to the examined specimens. The one-way ANOVA on the 'malabaricus-altus dataset' reveals significant size differences in log-transformed standard length ($F = 6.721$, $p = 0.0015$; Fig. 8B). The Tukey HSD post hoc test shows significant size differences between *D. malabaricus* and *D. altus* sp. nov. ($p = 0.002$) but not between *D. malabaricus* and *D.* cf. *malabaricus* ($p=0.105$) or between *D. altus* sp. nov. and *D.* cf. *malabaricus* ($p=0.065$).

In the PCA analysis of log-transformed, size-corrected morphometric and meristic 'malabaricus-altus dataset', PC1 and PC2 collectively accounted for 33.5% of the total variance (Suppl. material 5). The PC1 vs PC2 plot revealed that *D.* cf. *malabaricus* clustered closer to *D. malabaricus* than to *D. altus* sp. nov. in morpho space along both the PC1 and the PC2 axes (Fig. 9B). The species differed along the major axis of morphological variation in PC1, explaining 18.4% of the total variation (Fig. 9B; 'species' [$F= 107.80$, $p<0.0001$]); and PC2, explaining 15.2% of the total variation (Fig. 9B; 'species' [$F= 18.29$, $p<0.0001$]).

The frequency of the presence or absence of the infraorbital process in the 'malabaricus-altus dataset' is shown in Fig. 10B. With increasing elevation, the frequency of occurrence of the infraorbital process too, increases (Fig. 11). The infraorbital process is present in 25 while absent in 50 of the specimens of *D.* cf. *malabaricus*. The differences in the presence or absence of the infraorbital process between *D. malabaricus*, *D. altus* sp. nov., and *D.* cf. *malabaricus* is statistically significant based on Fisher's Exact Test ($p = 8.182e\text{-}11$).

The variation of the ratio of the length of the l+1 interstripe to standard length ([I+1]/SL) is statistically significant ($F= 10.87$, $p= 4.04e\text{-}05$) in the 'malabaricus-altus dataset' (Fig. 12A). The Tukey HSD post hoc test shows significant differences between *D. malabaricus* and *D. altus* sp. nov. ($p=0.0060$), and *D. malabaricus* and *D.* cf. *malabaricus* ($p=0.0001$), but not between *D. altus* sp. nov. and *D.* cf. *malabaricus* ($p=0.8640$). The I+1 interstripe is longer in examples from higher elevations than those from the lowlands (Figs 11, 12).

The P-stripe is bifurcated anteriorly in 46 and not bifurcated in 25 specimens of *D. malabaricus* (Fig. 12B). In contrast, there is no anterior bifurcation of the P-stripe in any of the specimens of *D. altus* sp. nov. or *D.* cf. *malabaricus*. This difference is statistically significant based on Fisher's Exact Test ($p < 2.2e\text{-}16$). The frequency of the anterior bifurcation of the P-stripe is more apparent and frequent in the samples of *D. malabaricus* from the lowlands compared to those from the highlands (Figs 11, 12).

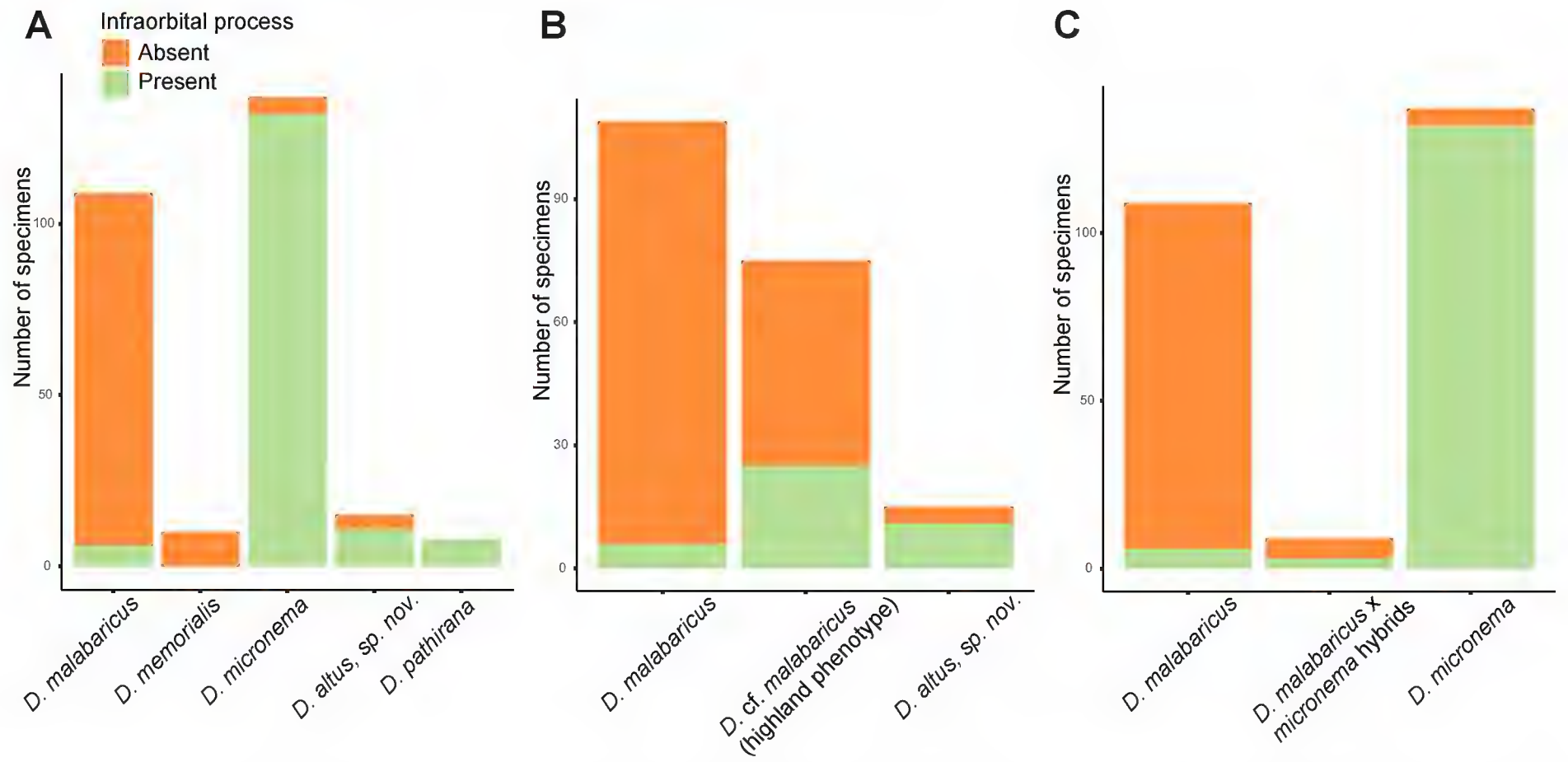

Figure 10. Variation of the presence or absence of the infraorbital process in **A.** The 'species dataset' (n=279); **B.** The 'malabaricus-altus dataset' (n=199); **C.** The 'malabaricus-micronema-hybrid dataset' (n=255).

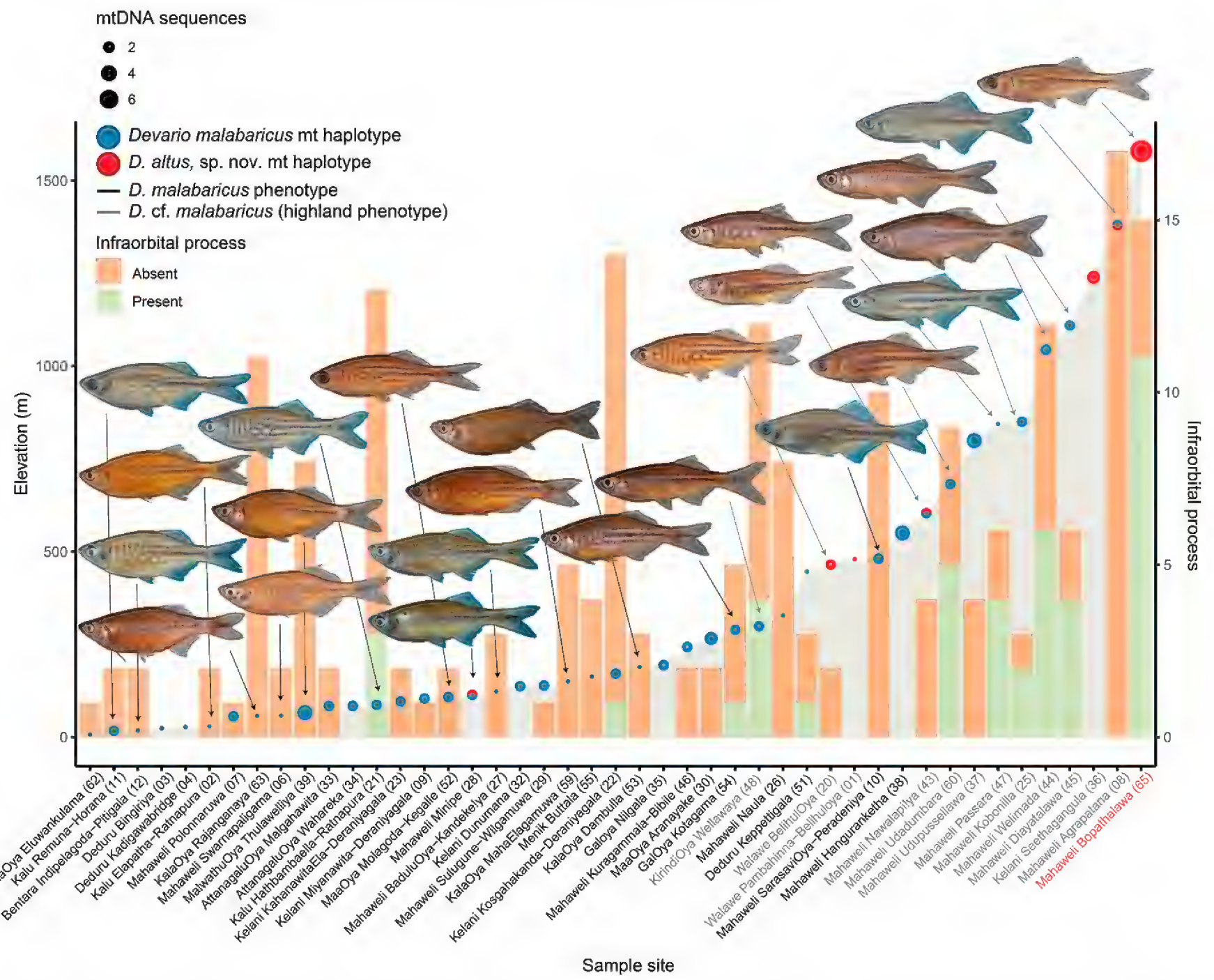

Figure 11. Phenotype and mitochondrial haplotype variation along the elevation gradient in *Devario malabaricus*, *D.* cf. *malabaricus* (highland phenotype), and *D. altus* sp. nov. The mitochondrial haplotype corresponding to *D. malabaricus* is in blue and the mitochondrial haplotype corresponding to *D. altus* sp. nov. is in red. The areas of the circles are proportional to the number of individuals sharing a given haplotype. Localities along the x axis in black represent phenotypes identified as *D. malabaricus* while those in grey represent phenotypes identified as *D.* cf. *malabaricus* (highland phenotype). The type locality of *D. altus* sp. nov. is in red. Numbers in parenthesis represent the sampling localities listed in Table 1. The frequency of presence or absence of the infraorbital process in specimens examined at each locality is shown as a bar plot.

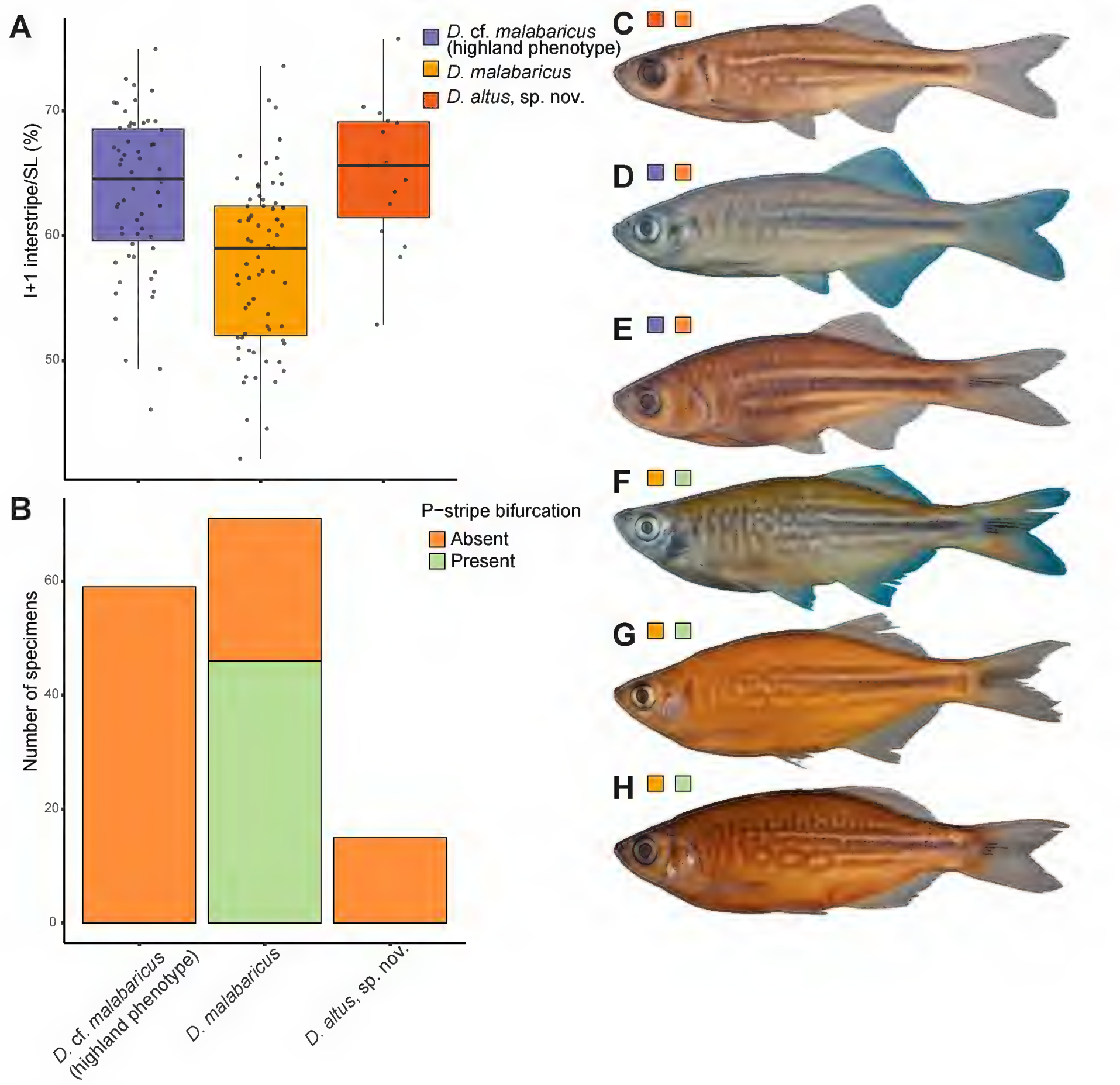

Figure 12. A. Variation of the proportion {I+1 interstripe}/standard length; **B.** Whether P-stripe is bifurcated anteriorly or not for a subset (n=145) of the 'malabaricus-altus dataset'. Specimen coloration of **C.** *Devario altus* sp. nov.; **D, E.** *D.* cf. *malabaricus* (highland phenotype); **F–H.** *D. malabaricus*.

Morphological analysis: the 'malabaricus-micronema-hybrid dataset'

Suppl. material 6 provides the morphometric and meristic data for the specimens examined. The one-way ANOVA of the 'malabaricus-micronema-hybrid dataset' reveals significant size differences in log-transformed SL ($F = 19.14$, $p = 1.83e\text{-}08$; Fig. 8C). The Tukey HSD post hoc test shows significant size differences between *D. micronema* and *D. malabaricus* ($p < 0.001$), but not between *D. malabaricus* and hybrids ($p = 0.253$) or between *D. micronema* and hybrids ($p = 0.760$).

In the PCA analysis of log-transformed, size-corrected morphometric and meristic data for the 'malabaricus-micronema-hybrid dataset', PC1 and PC2 collectively accounted for 24.9% of the total variance (Suppl. material 7). The PC1 vs PC2 plot revealed that the hybrid samples are clustered between *D. malabaricus* and *D. micronema* along both PC1 and PC2 (Fig. 9C). There were no significant differences along PC1, which explained 14.4% of the total variation (Fig. 9C; 'species' [$F = 2.30$, $p = 0.204$]). However, differences were significant along PC2, explaining 10.4% of the total variation (Fig. 9C; 'species' [$F = 63.44$, $p<0.0001$]).

The frequency of the infraorbital process in the 'malabaricus-micronema-hybrid dataset' is shown in Fig. 10C. Among the 9 specimens putatively considered hybrids, the process is present in only 3. The differences in the presence or absence of the infraorbital process between *D. malabaricus*, *D. micronema*, and hybrids is statistically significant based on Fisher's Exact Test ($p < 2.2e\text{-}16$).

Taxonomic accounts

Devario altus sp. nov.

Fig. 13A–C

https://zoobank.org/AAB6FB30-AAEA-4A8E-A4E4-ABA3064D0121

Devario monticola Batuwita, de Silva & Udugampala, 2017 (in part).

Type materials. ***Holotype.*** 2025.04.01.NH, 48.7 mm SL, Bopathalawa, Menik Palama Farm, Agra Oya (Mahaweli basin), 6°48'56"N, 80°42'23"E, 1580 m asl, H. Sudasinghe, 14 June 2019.

Paratypes. 2025.04.02.NH, 1, 44.2 mm SL (same data as holotype); DZ5260, 13, 41.9–54.9 mm SL (same data as holotype).

Diagnosis. *Devario altus* sp. nov. is distinguished from its Sri Lankan congeners by the combination of the following characters and character states: anal-fin origin posterior to dorsal-fin origin, beneath 3rd branched dorsal-fin ray; 1st infraorbital usually bearing a laterally-directed process; P-stripe continuous, not bifurcated anteriorly; interstripe I+1 long, extending uninterrupted anterior to vertical through pelvic-fin origin; caudal peduncle long, 19.2–21.4% SL, shallow, 8.8–10.5% SL; body shallow, its depth 21.8–26.0% SL; dorsal-fin height 19.0–23.2% SL; dorsal-fin base length 15.1–18.2% SL; anal-fin base length 16.4–20.5% SL; pelvic fin height 12.9–16.6% SL; pectoral-fin height 20.3–23.4% SL; head depth 17.3–19.6% SL; scales between lateral line and dorsal-fin origin 6–7½; circumpeduncular scales, 13–14; branched dorsal-fin rays 8½-10½.

Specifically, *D. altus* sp. nov. differs from *D. malabaricus* by possessing (in 73 percent of specimens) a process on the 1st infraorbital vs lacking (in 95 percent of *D. malabaricus*); having the P-stripe continuous, not bifurcated anteriorly (vs P-stripe usually bifurcated anteriorly); interstripe I+1 long, extending uninterrupted anterior to vertical through pelvic-fin origin (vs short, not extending uninterrupted anterior to a vertical through the pelvic-fin origin in *D. malabaricus*); a caudal peduncle length of 19.2–21.4% SL (vs 12.3–19.4% SL); a body depth of 21.8–26.0% SL (26.1–35.4% SL); and scale rows between lateral line and dorsal-fin origin 6–7½ (vs 7½-9).

The new species is distinguished from *D. memorialis* by having the anal-fin origin beneath the 3rd branched dorsal-fin ray, distinctly posterior to dorsal-fin origin (vs on vertical through dorsal-fin in *D. memorialis*); the anal-fin base length 16.4–20.5% SL (vs 20.0–23.4% SL); a head depth of 17.3–19.6% SL (vs 14.1–15.8% SL); and 13 or 14 (vs 15–18) circumpeduncular scales.

Devario altus sp. nov. differs from *D. micronema* by having the I+1 interstripe long, extending uninterrupted anterior to a vertical through the pelvic-fin origin (vs short, not extending uninterrupted anterior to a vertical through the pelvic-fin origin in *D. micronema*); having the P-stripe continuous, uninterrupted anteriorly (vs P-stripe usually interrupted anterior to pelvic-fin origin with bars or blotches); having a longer caudal peduncle, 19.2–21.4% SL (13.2–20.0% SL); having a body depth of 21.8–26.0% SL (vs 23.5–36.6% SL); and having 6–7½ (7½-9½) scale rows between lateral line and dorsal-fin origin.

It is distinguished from *Devario pathirana* by a shallower body (21.8–26.0% SL, vs 32.4–36.9% SL in *D. pathirana*); a shallower caudal peduncle depth of 8.8–10.5% SL (vs 11.3–13.1% SL); a shorter dorsal-fin base (15.1–18.2% SL, vs 19.0–23.8% SL); a shorter anal-fin base (16.4–20.5% SL, vs 20.7–22.8% SL); a shorter pelvic fin (12.9–16.6% SL, vs 16.1–18.8% SL); a shorter pectoral fin (pectoral-fin height 20.3–23.4% SL, vs 23.8–27.9% SL); 6–7½ (vs 8–8½) scale rows between lateral line and dorsal-fin origin; and 8½-10½ (vs 11½) branched dorsal-fin rays.

Devario altus sp. nov. is genetically distinct from *D. malabaricus*, its closest congener, by 1.2–1.7% in the *cox1* DNA barcoding marker and genetically distinct from the remaining species of Sri Lankan *Devario* by more than 3.6%. Some populations of *Devario* in the central hills ('*D.* cf. *malabaricus* (highland phenotype)') possess a color pattern resembling that of *D. altus* sp. nov.: however, some of the individuals sequenced from these populations contain the mitochondrial haplotype for *D. altus* sp. nov., while most others contain the *D. malabaricus* mitochondrial haplotype. Sexual dimorphism is apparent in *D. altus* sp. nov., with males possessing (and females lacking) tubercles on the branched rays of the pectoral fin.

Description. For general appearance, see Fig. 13A–C; morphometric data are provided in Suppl. material 2. Head and body laterally compressed, elongate. Body depth greatest at pelvic-fin origin. Snout length subequal to eye diameter. Medial margins of dentaries straight, parallel, with an indentation ('danionine notch') anteriorly. Well-developed dermal grooves present along supraorbital shelves. Small, rounded, symphysial knob present, fitting into shallow groove on inner margin of upper jaw. Lower jaw slightly longer than upper. First infraorbital with a process (n=11) or smooth (n=4). Tubercles on lower jaw rounded, arranged in a mediodorsal band of 2–3 rows, tapering to a single row posteriorly and towards symphysis, present in both sexes. Single row of rounded tubercles on upper margin of upper jaw. Pectoral-fin tubercles present in males. Both maxillary and rostral barbels present. Rostral barbel longer than maxillary barbel, not reaching anterior margin of orbit; maxillary barbel short, not reaching anterior margin of orbit. Lateral line complete, declining steeply for first 7 scales, then curving parallel to ventral body outline, running low on caudal peduncle, terminating on caudal-fin base, with 36 (2), 37 (6), 38 (3), 39 (1), 40 (2), or 41 (1) pored scales on body, plus 1–2 on caudal-fin base. Median predorsal scales 15 (3), 16 (10), 17 (1), or 18 (1). Lateral scale rows between origins of dorsal and pelvic fins 6+1+1 (2), ½6+1+1 (6), 7+1+1 (2), or ½7+1+1 (5). Circumpeduncular scales 13 (7) or 14 (8). Dorsal fin with 2–3 unbranched rays, 8½ (1), 9½ (10), or 10½ (4) branched rays, origin anterior to ver-

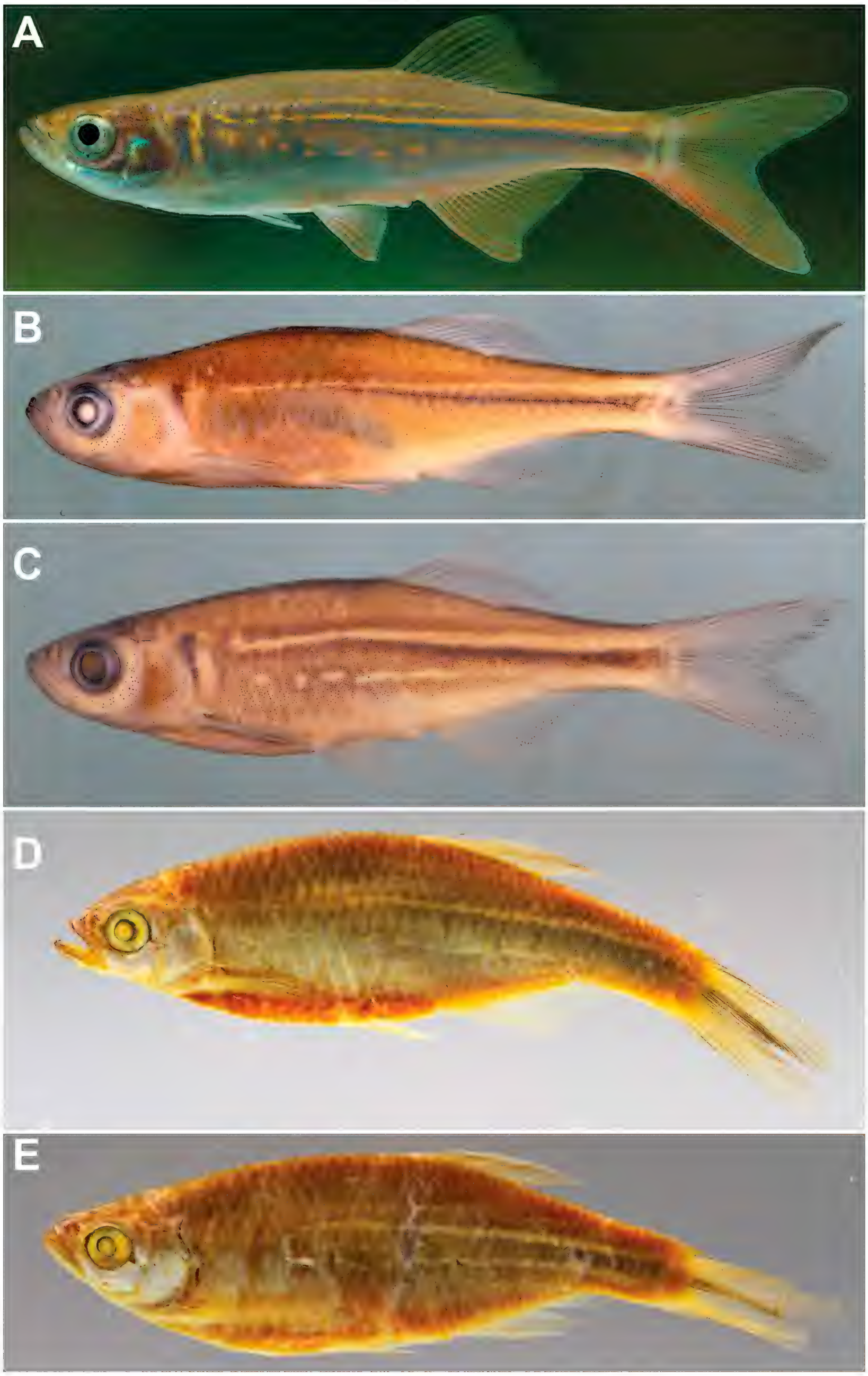

Figure 13. Coloration of *Devario altus* sp. nov., **A.** In aquarium (~50 mm SL); **B.** In preservative, holotype, 2025.04.01.NH, 48.7 mm SL; **C.** Paratype, DZ5260C, 52.1 mm SL. Bopathalawa, Menik Palama Farm, Agra Oya (Mahaweli basin), Sri Lanka. Holotype of **D.** *Danio micronema* Bleeker, 1863a, RMNH.PISC.2746A, 60.0 mm SL, Ceylon; and syntype of **E.** *Danio lineolatus* Bleeker, 1863a, RMNH.PISC.2746B, 59.2 mm SL, Ceylon.

tical through anal-fin origin, distal margin straight. Anal fin with 3 unbranched rays, 12½ (5), 13½ (7), 14½ (2), or 15½ (1) branched rays, distal margin slightly concave. Pectoral-fin with 1 unbranched and 9 (5), 10 (8) or 11 (2) branched rays, adpressed fin just reaching or surpassing pelvic-fin origin; axial lobe well developed. Pelvic fin with 1 unbranched and 6 (6), or 7 (9) branched rays, origin midway between anal-fin origin and pectoral-fin origin; tip of adpressed pelvic fin not reaching anal-fin origin. Pelvic 'axillary' scale present. Caudal fin with 9+8 (15) branched rays, forked, lobes subequal, rounded distally.

Color pattern. For condition in life, see Fig. 13A; in preservative, see Fig. 13B, C. In preservative, mid-dorsal stripe narrow, extending from occiput to caudal peduncle. Cleithral spot vertically elongate, covering part of first lateral-line scale and scale above it. P-stripe broad, two scale-widths wide anteriorly, 1 scale-width wide posteriorly, continuing to end of medial caudal-fin rays; anteriorly uninterrupted above pectoral and pelvic fins, bordered dorsally and ventrally by narrow I+1 and I, respectively. Interstripe I narrow, of uniform width, about ¼-⅓ width of P-stripe, slightly narrow to P-1, slightly narrower than I+1. Interstripe I+1 narrower anteriorly, about ½-⅓ width of P-stripe, wider posteriorly, subequal to P-stripe, uninterrupted anterior to pelvic-fin origin. P+1 originating above middle of pectoral fin, narrower than P, subequal I+1 anteriorly, tapering to caudal peduncle, not reaching caudal-fin base. Interstripe I+2 absent. P+2 absent. P-1 narrower than P, faint, with scattered melanophores, tapering to caudal peduncle, not reaching caudal-fin base; I-1, P-2 absent. Anterior bars indistinct or absent. Dorsal and anal fins hyaline, with light scattering of small melanophores along interradial membranes. Caudal-fin rays pigmented anteriorly; both rays and interradial membranes pigmented posteriorly. Pectoral and pelvic fins hyaline.

Habitat and distribution. Among the localities sampled in the central hills, the only one in which the mitochondrial genotype consists solely of *D. altus* is Bopathalawa, at 1580 m asl, the highest elevation at which any species of *Devario* has been recorded in Sri Lanka. Here, the 1–2 m-wide stream traverses Manik Palama, a dairy farm. The only other fish recorded here was the exotic *Poecilia reticulata* Peters, 1859.

Etymology. The species-name *altus* is Latin for 'high', an allusion to this species being restricted to the Sri Lankan highlands. Applied as a noun in apposition. Suggested vernacular name in English: Sri Lanka Montane Danio.

Devario malabaricus (Jerdon, 1849)

Perilampus malabaricus Jerdon, 1849.
Devario monticola Batuwita, de Silva & Udugampala, 2017.
Danio lineolatus Bleeker, 1863a.

Material examined. 109 ex., see Suppl. material 1.

Diagnosis. *Devario malabaricus* is distinguished from Sri Lankan congeners by the combination of the following characters and character states: anal-fin origin posterior to dorsal-fin origin, beneath 3rd branched dorsal-fin ray (vs anal-fin origin on vertical through dorsal-fin origin in *D. memorialis*); 1st infraorbital smooth, with no infraorbital process (vs present in *D. micronema*, *D. pathirana*, and most specimens of *D. altus*); P-stripe usually bifurcated anteriorly (vs P-stripe not bifurcated in *D. altus* and *D. memorialis*); interstripe I+1 short, usually not extending uninterrupted anterior to vertical through pelvic-fin origin (vs long, extending uninterrupted anterior to vertical through pelvic-fin origin in *D. altus*); caudal peduncle length 12.3–19.4% SL (vs 19.2–21.4% SL in *D. altus*); caudal peduncle depth 9.8–12.9% SL (vs 8.1–9.8% SL in *D. memorialis*); body depth 26.1–35.4% SL (vs 21.8–26.0% SL in *D. altus*; 15.9–22.6% SL in *D. memorialis*); dorsal-fin base length 16.7–23.5% SL (vs 13.5–16.3% SL in *D. memorialis*); pelvic-fin height 13.3–18.1% SL (vs 11.6–13.3% SL in *D. memorialis*); 33–39 pored lateral-line scales (vs 38–46 in *D. memorialis*); predorsal scales 14–18 (vs 17–19 in *D. memorialis*); 12–15 circumpeduncular scales (vs 15–18 in *D. memorialis*); 7½-9 scales between lateral line and dorsal-fin origin (vs 7½ in *D. memorialis*; 6–7½ in *D. altus*); 9½-12½ branched dorsal-fin rays (vs 8½ in *D. memorialis*).

Devario malabaricus is genetically distinct from *D. altus*, its closest congener, by 1.2–1.7% in the *cox1* DNA barcoding marker, and from the remaining Sri Lankan species of *Devario* by more than 2.8%. Sexual dimorphism is apparent in *D. malabaricus*, with the branched rays of the pectoral fin exhibiting tubercles in males but not in females (Sudasinghe 2024).

Habitat and distribution. *Devario malabaricus* is the only non-endemic member of the genus in Sri Lanka: it occurs also in peninsular India. It is the most widespread species of *Devario* on the island, a generalist that utilizes lotic and lentic, shaded and unshaded, clear and turbid, and disturbed and undisturbed habitats, from lowland floodplains to hill streams and torrents.

Remarks. Kullander et al. (2017) and Sudasinghe and Pethiyagoda (2019) showed that the neotype designation for *Perilampus malabaricus* Jerdon, 1849 by Batuwita et al. (2017) is inconsistent with Article 75.3.1 of the International Code of Zoological Nomenclature and hence, invalid. Although *Devario malabaricus* lacks a name-bearing type, it is common in its type locality, Malabar, which is unambiguously defined in contemporaneous maps. While Indian samples of *D. malabaricus* were not included in the present analysis, previous work (Batuwita et al. 2017; Sudasinghe et al. 2020a) has shown through both morphological and genetic analysis, that the Indian and Sri Lankan populations are conspecific.

Devario memorialis Sudasinghe, Pethiyagoda & Meegaskumbura, 2020a

Devario memorialis Sudasinghe, Pethiyagoda & Meegaskumbura, 2020a.

Material examined. 10 ex., see Suppl. material 1.

Diagnosis. *Devario memorialis* is distinguished from its Sri Lankan congeners by the combination of the following characters and character states: anal-fin origin on vertical through dorsal-fin origin (vs anal-fin origin posterior to dorsal-fin origin, beneath 3rd branched dorsal-fin ray in *D. malabaricus*, *D. micronema*, *D. altus*, and *D. pathirana*); 1st infraorbital smooth, with no infraorbital process (vs infraorbital process present in *D. micronema*, *D. pathirana*, and most specimens of *D. altus*); anterior bars on body absent or indistinct (vs present in *D. malabaricus*, *D. micronema*, and *D. pathirana*); preanal length 58.5–63.8% SL (vs 64.9–69.8% SL in *D. pathirana*); caudal peduncle depth 8.1–9.8% SL (vs 9.8–12.9% SL in *D. malabaricus*, 11.3–13.1% SL in *D. pathirana*); body depth 15.9–22.6% SL (vs 26.1–35.4% SL in *D. malabaricus*, 23.5–36.6% SL in *D. micronema*, 21.8–26.0% SL in *D. altus*, 32.4–36.9% SL in *D. pathirana*); dorsal-fin height 15.7–20.0% SL (vs 19.0–23.2% SL in *D. altus*, 20.6–24.3% SL in *D. pathirana*); dorsal-fin base length 13.5–16.3% SL (vs 16.7–23.5% SL in *D. malabaricus*, 16.6–23.3% SL in *D. micronema*, 19.0–23.8% SL in *D. pathirana*); anal-fin base length 20.0–23.4% SL (vs 16.4–20.5% SL in *D. altus*); pelvic-fin height 11.6–13.3% SL (vs 13.3–18.1% SL in *D. malabaricus*, 13.8–18.5% SL in *D. micronema*, 12.9–16.6% SL in *D. altus*, 16.1–18.8% SL in *D. pathirana*); pectoral-fin height 19.1–23.6% SL (vs 23.8–27.9% SL in *D. pathirana*); head depth 14.1–15.8% SL (vs 16.0–23.0% SL in *D. micronema*, 17.3–19.6% SL in *D. altus*, 17.6–21.7% SL in *D. pathirana*); interorbital width 37.2–40.7% HL (vs 40.7–45.3% HL in *D. pathirana*); rostral barbel 4.0–8.9% HL (vs 8.3–14.2% HL in *D. altus*, 8.6–17.7% HL in *D. pathirana*); 38–46 lateral-line scales (vs 33–39 in *D. malabaricus*); predorsal scales 17–19 (vs 14–18 in *D. malabaricus*, 13–17 in *D. micronema*, 14–15 in *D. pathirana*); 15–18 circumpeduncular scales (vs 12–15 in *D. malabaricus*, *D. micronema*, 13–14 in *D. altus*, *D. pathirana*); 7½ scales between lateral line and dorsal-fin origin (vs 7½–9 in *D. malabaricus*, 7½–9½ in *D. micronema*, 6–7½ in *D. altus*, 8–8½ in *D. pathirana*); 8½ branched dorsal-fin rays (vs 9½–12½ in *D. malabaricus*, *D. micronema*, 8½–10½ in *D. altus*, 11½ in *D. pathirana*); 14½–15½ branched anal-fin rays (vs 13½–14½ in *D. pathirana*).

Devario memorialis is genetically distinct from the remaining Sri Lankan species of *Devario* by an uncorrected p-distance of more than 3.9% in the *cox1* DNA barcoding marker.

Habitat and distribution. *Devario memorialis* has been recorded only from a 3-kilometer section of a headwater tributary of the Ma Oya (river) near Aranayake, at an elevation of 238–266 m above sea level. This species typically inhabits the fast-flowing sections of the stream.

Remarks. At some of the sampling sites at Aranayake, *D. malabaricus* occurs in syntopy with *D. memorialis* but at a lower frequency than the latter. All the specimens dissected to establish sex were females. Pectoral-fin tuberculation is absent in all the specimens in our series: it is possible that pectoral-fin tuberculation is absent in both sexes of *D. memorialis*.

Devario micronema (Bleeker, 1863a)

Danio micronema Bleeker, 1863a.

Devario annnataliae Batuwita, de Silva & Udugampala, 2017.

Devario udenii Batuwita, de Silva & Udugampala, 2017.

Material examined. 137 ex., see Suppl. material 1.

Diagnosis. *Devario micronema* is distinguished from Sri Lankan congeners by the combination of the following characters and character states: anal-fin origin posterior to origin of dorsal fin, beneath 3rd branched dorsal-fin ray (vs anal-fin origin on vertical through dorsal-fin origin in *D. memorialis*); 1st infraorbital with an infraorbital process (vs infraorbital process absent in *D. malabaricus* and *D. memorialis*); interstripe I+1 short, usually not extending uninterrupted anterior to vertical through pelvic-fin origin (vs long, extending uninterrupted anterior to pelvic-fin origin in *D. altus*); caudal peduncle length 13.2–20.0% SL (vs 19.2–21.4% SL in *D. altus*); body depth 23.5–36.6% SL (vs 15.9–22.6% SL in *D. memorialis*); dorsal-fin base length 16.6–23.3% SL (vs 13.5–16.3% SL in *D. memorialis*); pelvic-fin height 13.8–18.5% SL (vs 11.6–13.3% SL in *D. memorialis*); head depth 16.0–23.0% SL (vs 14.1–15.8% SL in *D. memorialis*); predorsal scales 13–17 (vs 17–19 in *D. memorialis*); circumpeduncular scales 12–15 (vs 15–18 in *D. memorialis*); scales between lateral line and dorsal-fin origin 7½-9½ (vs 7½ in *D. memorialis*); branched dorsal-fin rays 9½-12½ (vs 8½ in *D. memorialis*).

Devario micronema is genetically distinct from *D. pathirana*, its closest congener by 1.2–1.6% in the *cox1* DNA barcoding marker, and from the remaining Sri Lankan species of *Devario* by over 2.8%. Among these, *D. micronema* shows, across its range, the greatest phenotypic diversity in terms of color-pattern and habitus (Fig. 14) as well as greatest genetic diversity among the Sri Lankan species of *Devario*. Several of our specimens are suggestive of mitonuclear discordance and/or phenotypes that are intermediate between *D. malabaricus* and *D. micronema*. These specimens are considered in this study as potential hybrids. Sexual dimorphism in *D. micronema* is apparent, with males exhibiting tubercles on the branched rays of the pectoral fin, a character absent in females (Sudasinghe 2024).

Habitat and distribution. Among the endemic species of *Devario*, *D. micronema* is the most widely distributed giant danio in Sri Lanka. It is recorded from clear, shaded, undisturbed, low-elevation rainforest streams in the per-humid southwestern wet zone, from sea level to about 500 m asl in Kelani, Kalu, Bentara, and Gin River basins.

Remarks. The Sri Lankan fishes described by Bleeker (1863a) have not been reported on since their original description: they were presumed lost. The collection was, however, located among uncatalogued material in RMNH by Martien J P. Van Oijen and Maurice Kottelat ca 2015 (Sudasinghe et al. 2025). One of us (HS) examined the holotype of *D. micronema*, a 60.0 mm SL specimen (Fig. 13D). This is entirely consistent with our conception of the species based on the material examined here.

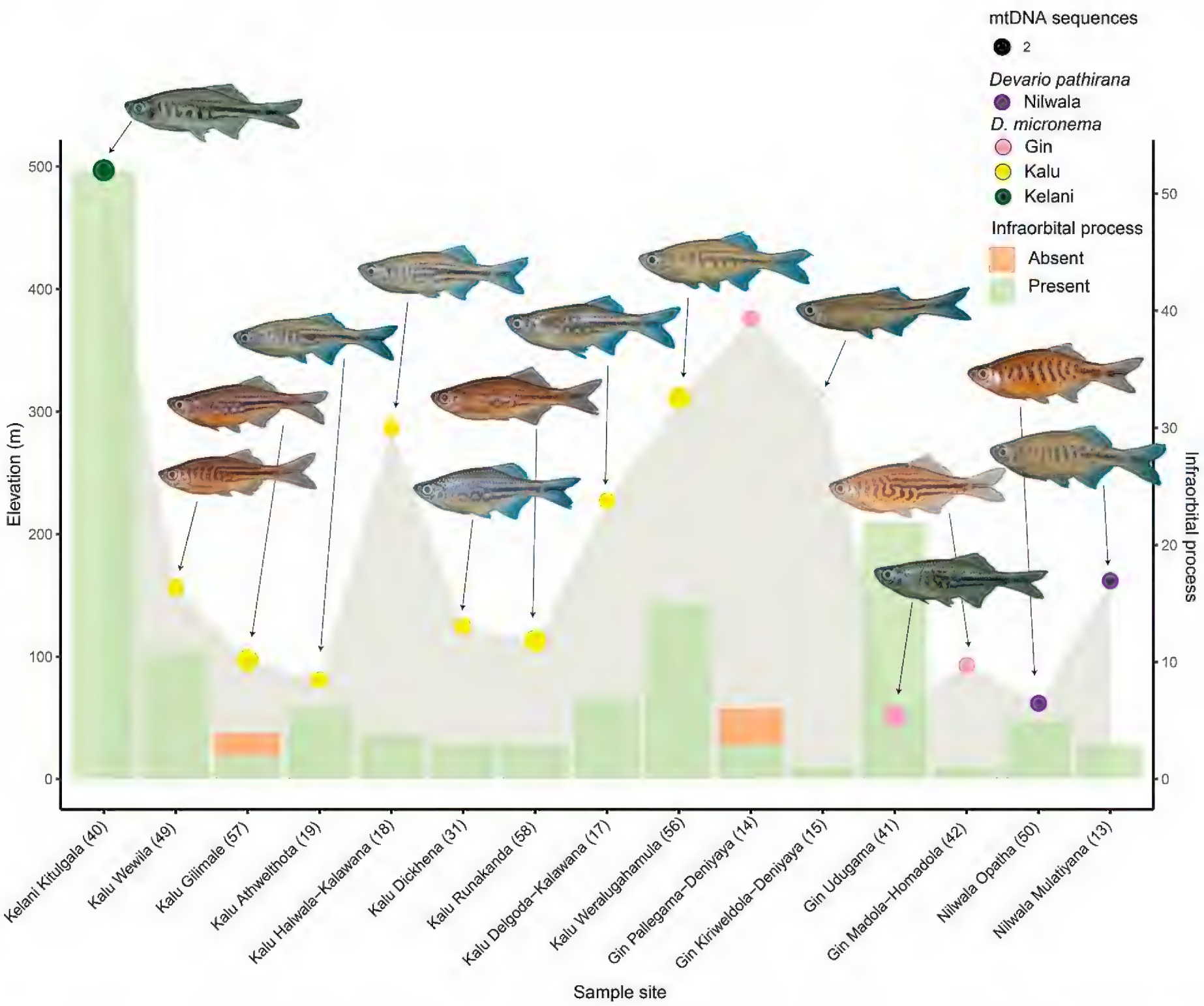

Figure 14. Phenotype and mitochondrial haplotype variation along the elevation gradient in *Devario micronema* and *D. pathirana*. The areas of the circles are proportional to the number of individuals sharing a given haplotype. Sampled localities are on the x axis; numbers in parenthesis represent the sampling localities as listed in Table 1. The frequency of the presence or absence of the infraorbital process in specimens examined at each locality is shown as a bar plot.

Devario pathirana (Kottelat & Pethiyagoda, 1990)

Danio pathirana Kottelat & Pethiyagoda, 1990.

Material examined. 8 ex., see Suppl. material 1.

Diagnosis. *Devario pathirana* is distinguished from Sri Lankan congeners by the combination of the following characters and character states: it is the only completely barred *Devario* in the island, with 7–11 bars on the side of the body (vs fewer than 4 bars in *D. malabaricus*, *D. micronema*, *D. memorialis*, *D. altus*); P-stripe short, ending above the caudal peduncle (P-stripe long, extending uninterrupted above the caudal peduncle in *D. malabaricus*, *D. micronema*, *D. memorialis*, *D. altus*); anal-fin origin posterior to origin of dorsal fin, beneath 3rd branched dorsal-fin ray (vs anal-fin origin on vertical through dorsal-fin origin in *D. memorialis*); 1st infraorbital with an infraorbital process (vs infraorbital process absent in *D. malabaricus*, *D. memorialis*); preanal length 64.9–69.8% SL (vs 58.5–63.8% SL in *D. memorialis*); caudal peduncle length 15.7–19.6% SL (vs 19.2–21.4% SL in *D. altus*); caudal peduncle depth 11.3–13.1% SL (vs 8.1–9.8% SL in *D. memorialis*, 8.8–10.5% SL in *D. altus*); body depth 32.4–36.9% SL (15.9–22.6% SL in *D. memorialis*; 21.8–26.0% SL in *D. altus*); dorsal-fin height 20.6–24.3% SL (vs 15.7–20.0% SL in *D. memorialis*); dorsal-fin base length 19.0–23.8% SL (vs 13.5–16.3% SL in *D. memorialis*, 15.1–18.2% SL in *D. altus*); anal-fin base length 20.7–22.8% SL (vs 16.4–20.5% SL in *D. altus*); pelvic-fin height 16.1–18.8% SL (vs 11.6–13.3% SL in *D. memorialis*; 12.9–16.6% SL in *D. altus*); pectoral-fin height 23.8–27.9% SL (vs 19.1–23.6% SL in *D. memorialis*, 20.3–23.4% SL in *D. altus*); head depth 17.6–21.7% SL (vs 14.1–15.8% SL in *D. memorialis*); interorbital width 40.7–45.3% HL (vs 37.2–40.7% HL in *D. memorialis*); rostral barbel 8.6–17.7% HL (vs 4.0–8.9% HL in *D. memorialis*); 14–15 predorsal scales (vs 17–19 in *D. memorialis*); 13–14 circumpeduncular

scales (vs 15–18 in *D. memorialis*); 8–8½ scales between lateral line and dorsal-fin origin (vs 7½ in *D. memorialis*; 6–7½ in *D. altus*); 11½ branched dorsal-fin rays (vs 8½ in *D. memorialis*); 13½–14½ branched anal-fin rays (vs 14½–15½ in *D. memorialis*).

Devario pathirana is genetically distinct from *D. micronema*, its closest congener by 1.2–1.6% for the *cox1* DNA barcoding marker and genetically distinct from rest of the Sri Lankan species of *Devario* by over 3.6%.

Habitat and distribution. *Devario pathirana* is confined to the streams associated with the headwaters of the Nilwala River basin in the southwestern wet zone of Sri Lanka. The population of *Devario* in Suriyakanda, at the type locality of *Rasboroides rohani* in the Walawe basin, comprises of potential hybrids between *D. pathirana* and *D. malabaricus*. This is likely a result of an anthropogenic translocation (Sudasinghe et al. 2018).

Remarks. At some of the sampling sites in the headwaters of the Nilwala basin, both *D. malabaricus* and *D. micronema* were recorded along with *D. pathirana* (T. Ranasinghe, S. Wijayasekara pers. comm.). This suggests that there may be zones of contact between these species. Sexual dimorphism is apparent in *D. pathirana*, with males possessing tubercles on the branched rays of the pectoral fin, which are absent in females.

Notes on the taxonomy of *Devario monticola*, *D. annnataliae*, and *D. udenii*

Devario monticola. Sudasinghe and Pethiyagoda (2019) agreed with Batuwita et al. (2017) that *Danio lineolatus* is a secondary homonym in *Devario* of *Leuciscus lineolatus*. A replacement name was thus warranted, and Batuwita et al. (2017) supplied it, as *Devario monticola*. On the assumption that the name-bearing type of *D. monticola* was lost, these authors associated *D. monticola* with a montane population of *Devario* inhabiting the Agra Oya, a headwater tributary of the Mahaweli River, in the island's central hills. The name-bearing type of *Danio lineolatus* Bleeker, however, is present in RMNH: see Fig. 13E. Sudasinghe et al. (2025) treated *Danio lineolatus* (= *Devario monticola* Batuwita, de Silva & Udugampala, 2017), as a junior synonym of *Perilampus malabaricus* Jerdon, 1849. The population of *Devario* at Agra Oya described as *Devario monticola* by Batuwita et al. (2017) is referred to here as *D.* cf. *malabaricus* (highland phenotype), given that the samples sequenced from this locality contain a mix of *D. altus* and *D. malabaricus* mitochondrial genotypes.

Devario annnataliae. Sudasinghe and Pethiyagoda (2019) treated *Devario annnataliae* as a species inquirendum because the wealth of errors and inconsistencies in its original diagnosis and description made it impossible to distinguish it reliably from other species of *Devario*, especially from *D. micronema*. They also showed that the specimens accessioned in NMSL as the holotype and paratypes of *D. annnataliae* cannot be the specimens designated by Batuwita et al. (2017).

Batuwita et al. (2017) declared the type locality of *D. annnataliae* as "Gin River, Brahmana Ella falls in Sinharaja World Heritage Site, near Lankagama". Our molecular and morphological analysis includes two samples from Pallegama-Deniyaya and seven from Kiriweldola, which lie in the Gin basin less 12 km from the type locality of *D. annnataliae*. These examples are morphologically consistent with the description of the species by Batuwita et al. (2017) but fall within the clade we identify as *D. micronema*, at the same time showing no substantial morphological differences from specimens from across the range of this species. We therefore treat *Devario annnataliae* as a junior subjective synonym of *D. micronema*.

Devario udenii. Batuwita et al. (2017) also described another new species, *Devario udenii*. Sudasinghe and Pethiyagoda (2019) showed that this too was likely a junior synonym of *D. micronema*. Our morphological analysis, which includes topotypical specimens of this species, shows this indeed to be the case: *Devario udenii* is indistinguishable from *D. micronema*. Our molecular analysis includes topotypes of *D. udenii* which too, nest within the *D. micronema* clade. We therefore treat *Devario udenii* as a junior subjective synonym of *D. micronema*.

A key to the Sri Lankan species of *Devario*

1	Anal-fin origin on vertical through dorsal-fin origin	*D. memorialis*
–	Anal-fin origin posterior to dorsal-fin origin, beneath 3rd branched dorsal-fin ray	2
2	Only completely barred *Devario* in the island: 7–11 bars on the side of body; P-stripe short, interrupted above caudal peduncle	*D. pathirana*
–	Fewer than 4 bars on anterior side of body; P-stripe long, extending uninterrupted above caudal peduncle	3
3	P-stripe continuous, not bifurcated anteriorly; interstripe I+1 long, extending uninterrupted anterior to vertical through pelvic-fin origin	*D. altus* sp. nov.
–	P-stripe usually bifurcated anteriorly; I+1 interstripe short, not extending uninterrupted anterior to vertical through pelvic-fin origin	4
4	A laterally directed extension of the posterior edge of infraorbital 1 present	*D. micronema*
–	A laterally directed extension of the posterior edge of infraorbital 1 absent	*D. malabaricus*

Discussion

Phylogenetic relationships

The phylogenetic relationships of Sri Lankan *Devario* inferred from the mitochondrial DNA (mtDNA) dataset in the present study align closely with the findings of Sudasinghe et al. (2020a). Two major, well-supported clades of Sri Lankan *Devario* are evident. The first clade comprises lineages endemic to the island's southwestern wet zone: *D. memorialis*, *D. micronema*, and *D. pathirana*. Within this clade, *D. memorialis* is recovered as the sister group to a subclade containing *D. micronema* and *D. pathirana*. The second clade encompasses *D. malabaricus*, a species with a broad distribution across Sri Lanka and peninsular India, and *D. altus*, which is restricted to an elevation of around 1580 m asl in the central hills.

In contrast, the phylogenetic relationships inferred from the nuclear DNA (nuDNA) dataset are poorly resolved. The diversification of Sri Lankan *Devario* is a relatively recent event, having commenced during the Plio-Pleistocene (Sudasinghe et al. 2020a). Unsurprisingly given so shallow an evolutionary timescale, the two nuclear markers used in this study exhibit only weak phylogenetic signals (Chen et al. 2008; Bufalino and Mayden 2010). Although the phylogenetic network constructed here supports the clustering of distinct lineages, uncertainties persist in the connections among species. These ambiguities are likely attributable to hybridization events and/or incomplete lineage sorting. We suspect that genome-wide analyses using single-nucleotide polymorphisms (SNPs) could provide a more robust framework for resolving the associated phylogenetic relationships.

Recent studies have presented evidence for "Out of Sri Lanka" or Sri Lanka-to-India back-migration scenarios in freshwater fishes (Sudasinghe et al. 2023b, c). We suspect that a similar dynamic applies also to *Devario*. An ancestral lineage of *Devario* colonized Sri Lanka initially, diversified within the southwestern wet zone, and subsequently gave rise to *D. malabaricus*, which then migrated back to peninsular India. Testing this hypothesis will require comprehensive sampling of *Devario* populations across peninsular India, to reconstruct their evolutionary history more conclusively. This hypothesis is further supported by the whole of the Indian peninsula containing only three species: *D. malabaricus*, *Devario fraseri* (Hora & Mukerji, 1935) and *Devario neilgherriensis* (Day, 1867), the latter two of which are confined to the Western Ghats mountain range.

This scenario suggests that the back-migration of *Devario* to peninsular India occurred recently. Although several nominal species of *Devario*, in addition to *D. malabaricus* have been described from the Indian peninsula, including *Devario aurolineatus* (Day, 1865), *Devario canarensis* (Jerdon, 1849), *D. fraseri*, and *Devario mysoricus* (Jerdon, 1849), these taxa closely resemble the phenotype of *D. malabaricus*. A comprehensive taxonomic revision is therefore required to determine their validity and evolutionary relationships. The only lineage of *Devario* identified from peninsular India that is strikingly distinctive morphologically is *D. neilgherriensis*. This species is restricted to high elevations (up to 2200 m asl) in the Nilgiri Hills (Day 1867). A superficial comparison suggests that *D. neilgherriensis* exhibits a degree of morphological convergence with *D. altus*, including a slender body profile, an infraorbital process, and a similar coloration. These shared traits may represent an example of convergent evolution, driven by adaptation to hill-stream habitats in the two regions.

Phylogeography

The emerging phylogeographic patterns observed in Sri Lankan freshwater fishes suggest that habitat specialists, despite being restricted to smaller geographic ranges, exhibit stronger genetic structuring compared to habitat generalists, which have much broader distributions (Pethiyagoda and Sudasinghe 2021; Sudasinghe et al. 2023b). This pattern is further supported by the phylogeographic patterns of *D. malabaricus*, a habitat generalist species, and *D. micronema*, a habitat specialist species, revealed in the present study. In *D. micronema*, gene flow appears to be weaker even between adjacent river basins, resulting in pronounced genetic differentiation. In contrast, *D. malabaricus* shows no discernible genetic structuring, indicating extensive gene flow across its range.

The neutrality test results suggest contrasting demographic histories for the two species. For *D. malabaricus*, the significant negative Fu and Li's F* and negative Tajima's D indicate potential recent demographic expansion or selective sweeps, whereas for *D. micronema*, the non-significant neutrality test results are consistent with relatively stable populations and limited gene flow.

These observed phylogeographic patterns are critical for informing conservation management decisions, particularly in prioritizing species and habitats for protection, with a primary focus on the species assemblages of the southwestern wet zone, which are closely associated with rainforest ecosystems.

Mito-nuclear discordance

***Devario malabaricus* and *D. pathirana*.** Around 2003, several individuals of *Devario pathirana* had been illicitly translocated to Suriyakanda in the Walawe basin (Sudasinghe et al. 2018), to which *D. malabaricus* is native, but from which *D. pathirana* was until then absent. One of our samples from this location clusters with *D. pathirana* in the mtDNA phylogeny but aligns with *D. malabaricus* in the nuDNA phylogeny, while another clusters with *D. malabaricus* in the mtDNA phylogeny but aligns with *D. pathirana* in the nuDNA phylogeny. Phenotyp-

ically, however, these specimens (Fig. 1H) are more similar to *D. malabaricus* (Fig. 1F) than to *D. pathirana* (Fig. 1C). Sudasinghe et al. (2018) reported no specimens phenotypically resembling *D. pathirana* at this locality, leading them to conclude that the translocated population had failed to establish. However, our present results indicate that genetic material from *D. pathirana* has introgressed into the native *D. malabaricus* population at Suriyakanda.

This unintended experiment provides unambiguous evidence of hybridization and introgression between species of *Devario* in the wild, and the phenotypic consequences that result from it, such as in coloration and the presence/absence of the infraorbital process (Raesly et al. 1990; Hata et al. 2019; Blackwell et al. 2021; Ottenburghs 2021). The absence of evidence for hybridization between *D. malabaricus* and *D. pathirana* in the Nilwala basin, to which both species are native, is likely to be a result of insufficient sampling in their zone of natural contact. Based on our observations at Suriyakanda, we suspect that future investigations will reveal such hybrids to exist.

***Devario malabaricus* and *D. micronema*.** Evidence of hybridization between *D. malabaricus* and *D. pathirana* adds confidence to our hypothesis that natural hybrids between *D. malabaricus* (an open-habitat generalist) and *D. micronema* (a shaded-habitat rainforest specialist) occur in their zones of contact. At sites such as Andawaldola, Hiniduma, and Dellawa, such hybridization is putatively evidenced by mito-nuclear discordance (Fig. 3) and phenotypic traits that reflect a blend of characteristics from both species. In addition to coloration, these include variations in the presence or absence of the infraorbital process. Though originally within the rainforest, all three sites have undergone significant anthropogenic modification in recent decades. Such invasion by – and hybridization between – native species, giving rise to mito-nuclear discordance between them, is an illustrative example of a second-order effect of habitat modification (Esa et al. 2000; Sivyer et al. 2018; Ottenburghs 2021).

***Devario malabaricus* and *D. memorialis*.** Although both these species occur syntopically at Aranayake, to which the latter is restricted, our limited samples from their zone of contact did not reveal evidence of mito-nuclear discordance (Fig. 3). At these sites, *D. memorialis* was more abundant than *D. malabaricus*, and we suspect that the colonization of this habitat by the latter maybe recent, possibly associated with the loss of riparian shade. Genetically, this species pair is substantially divergent, with a 4.8% difference in the mitochondrial *cox1* gene. This degree of interspecific divergence is at the higher end of the spectrum for closely related *Devario* (Kullander et al. 2017; Sudasinghe et al. 2020a). Further, we speculate that the pectoral-fin tubercles in mature male *D. malabaricus* (*D. memorialis* is the only Sri Lankan *Devario* in which males appear to lack such tubercles) may be relevant to stimulating oviposition, possibly serving as a prezygotic barrier to hybridization between the two species.

***Devario malabaricus* and *D. altus*.** Because they lack a distinct zone of contact but vary as a cline between the lowland floodplain, where only *D. malabaricus* occurs, and the type locality of *D. altus* (1580 m asl, the elevational limit of the genus in Sri Lanka), interpreting mito-nuclear discordance in this species-pair is relatively more fraught. While the proportion of individuals that shows evidence of genetic discordance increases with elevation (Fig. 11), even among specimens showing no evidence of discordance, body depth and the frequency of the bifurcated P-stripe decrease. In contrast, the frequency of the infraorbital process increases as elevation increases. In this background, the question arises as to whether *D. altus* is a relictual species that has persisted in a highland refuge, or whether it represents the limit of adaptation by *D. malabaricus* to the highland environment.

Pethiyagoda and Sudasinghe (2021) argued from various strands of evidence that the freshwater fish fauna of the highlands had suffered one or more extinction events as recently as the late Pleistocene. Premathilake et al. (2003) showed, from pollen-core data at a site 10 km from the type locality of *D. altus*, that a desiccation event that lasted several thousand years straddled the Last Glacial Maximum. It is possible that *D. altus* was reduced to a headwater refugium at that time. The highlands were densely forested up to the British Colonial Period starting ca 1800, since when they were deforested almost in their entirety for the cultivation of coffee and tea. In the 1880s, rainbow trout (*Oncorhynchus mykiss*) were introduced to the highlands for a sport fishery that lasted until ca 1980, when the species was finally eradicated (except for a localized population in the headwaters of the Walawe River) (Pethiyagoda 2012). *Devario altus* may have persisted in its type locality because the stream in this shaded refuge is too shallow for trout to have invaded.

Such a highland refugial scenario for Sri Lankan freshwater fishes is further supported by the phylogeographic study of *Garra ceylonensis*, one of the few other native freshwater fishes inhabiting the Sri Lankan highlands (Sudasinghe et al. 2021). The highest elevation from which *Garra* were reported in that study was 1575 m asl, at Bopathalawa, just 2 km from the type locality of *D. altus*. This sample of *Garra* was highly divergent genetically from the other populations of *Garra* sampled at 14 locations in the elevation range 54–1083 m asl in the same (Mahaweli River) basin.

Deforestation (and hence the removal of riparian shade) and the eradication of rainbow trout, a notorious predator, may thus have facilitated a relatively recent invasion of the highlands by *Devario malabaricus*, with opportunities for hybridization with *D. altus*. Such a scenario is not necessarily confounded by our recording a specimen exhibiting mito-nuclear discordance at Minipe (100 m asl), in lower Mahaweli River. The water here derives from a cascade of reservoirs and hydro-

electric schemes that transfer water from the highlands, potentially accelerating the dispersal of introgressed individuals. While we currently consider *D. altus* to be restricted to the Mahaweli headwaters, the presence of *D. altus* genotypes in the Belihul Oya (Walawe Basin) and the headwaters of the Kelani Basin may reflect its historical distribution across the Central Highlands of Sri Lanka. The convergence of the Mahaweli, Kelani, Walawe, and Kirindi headwaters in this region makes it possible that headwater capture events gave rise to such a distribution. Indeed, headwater capture between the Mahaweli and adjacent basins is indicated by several Sri Lankan freshwater fishes (Sudasinghe et al. 2020b, c, d, 2021, 2023b). This context provides plausible explanations for the occurrence of *D. altus* genotypes in proximal regions despite the species' current confinement to the vicinity of Bopathalawa.

If *D. altus* is indeed the relictual species we believe it to be, it would imply that as *D. malabaricus* invaded the highlands, it replaced ancestral *D. altus* haplotypes with *D. malabaricus* haplotypes. Nevertheless, the alternative hypothesis posits that the observed mito-nuclear discordance could reflect local adaptation by *D. malabaricus*. This would mean that the '*D. altus* phenotype' reflects adaptation to the highlands by *D. malabaricus*. This can be argued by the existence of two distinct phenotypes in the *D. malabaricus* clade: the lowland one with greater body depth, the P-stripe bifurcated, shorter I+1 interstripe, and lacking an infraorbital process; and the highland one, with a shallower body, a P-stripe that is entire, longer I+1 interstripe, and with a greater likelihood of possessing an infraorbital process. In this scenario, *D. altus* and *D. malabaricus* would be taxonomically synonymous, representing a single species with phenotypic divergence driven by local adaptation. This presents an intriguing opportunity to test these alternative evolutionary hypotheses using comparative genomic and experimental approaches in the future.

Nevertheless, given that *D. altus* can unambiguously be distinguished phenotypically from *D. malabaricus* (see Diagnosis in species accounts, Suppl. material 2, and key, above), and is genetically distinct from *D. malabaricus*, its closest congener, by 1.2–1.7% in the *cox1* DNA barcoding marker (1.2–1.6% between *Devario pathirana* and *D. micronema*, 1.8% between *D. aequipinnatus* and *D. coxi*, 1.8% between *D. kakhienensis* and *D. fangae*, all of which are recognized as valid species: Kullander et al. 2017; Sudasinghe et al. 2020a), we consider *Devario altus* to be a valid species. It is also relevant that by the misnomer '*D. monticola*', this montane population of *Devario* is presently treated as Critically Endangered by the IUCN Red List (Goonatilake et al. 2020).

***Devario micronema* and *D. pathirana*.** Although no evidence of mito-nuclear discordance has been observed between these species, we note the occurrence of several populations in the Nilwala headwaters where phenotypes resembling both *D. pathirana* and *D. micronema* have been observed in syntopy (T. Ranasinghe, S. Wijayasekara, pers. comm.). These sites are proximal to the headwaters of the Gin Basin, to which *D. micronema* is native (*D. pathirana* is confined to the Nilwala River basin). This raises the question of whether these sites represent primary origins of divergence along the speciation spectrum or are the result of secondary colonization events by *D. micronema* following recent headwater capture events or anthropogenic hydrological alterations. Resolving such questions will require comprehensive sampling and the application of genomic tools to unravel the evolutionary history of these populations.

Among Sri Lankan *Devario*, populations of *D. micronema* exhibit substantial morphological variation in body shape and colour pattern, coupled with the greatest genetic diversity, even within small geographic areas (Fig. 14). This phenetic diversity could putatively be attributed to several factors. The ecological heterogeneity of the rainforest streams of the southwestern wet zone may impose varying selective pressures, driving local adaptations. Additionally, the topographical complexity of this region likely restricts gene flow between populations, is evident from our phylogeographic analysis (Fig. 6), giving rise to independent evolutionary trajectories. Such morphological diversification may also reflect an ongoing radiation within *D. micronema*, driven by ecological opportunities unique to Sri Lanka's freshwater ecosystems (Schluter 2000).

Further investigations integrating genomic tools with ecological and morphological analyses are necessary to elucidate the relative contributions of ecological factors to the genetic and phenotypic diversity observed in *D. micronema*. The present results provide a promising foundation based on which evolutionary dynamics in a novel tropical environment could be studied, contributing to our understanding of speciation and adaptation in diverse freshwater ecosystems.

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Supplementary material 1

Material of *Devario* examined in this study.

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Data type: docx

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Link: https://doi.org/10.3897/evolsyst.9.162251.suppl1

Supplementary material 2

Measurements (mm) and the effect of species and standard length on morphological traits for the 'species dataset' of *Devario*

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Data type: docx

Explanation note: Values in bold for morphological traits represent non-overlapping range while values in bold for effect represent significance at $P < 0.05$ even after Bonferroni correction.

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Supplementary material 3

Component loadings in the principal component analysis of morphometric measurements and meristics for the 'species dataset' of *Devario*

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Data type: docx

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Supplementary material 4

Measurements (mm) of morphological traits for the 'malabaricus-altus dataset' of *Devario*

Authors: Hiranya Sudasinghe, Rohan Pethiyagoda, Madhava Meegaskumbura

Data type: docx

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Supplementary material 5

Component loadings in the principal component analysis of morphometric measurements and meristics of the 'malabaricus-altus dataset' of *Devario*

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Data type: docx

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Supplementary material 6

Measurements (mm) of morphological traits of the 'malabaricus-micronema-hybrid dataset' of *Devario*

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Supplementary material 7

Component loadings in the principal component analysis of morphometric measurements and meristics of the 'malabaricus-micronema-hybrid dataset' of *Devario*

Authors: Hiranya Sudasinghe, Rohan Pethiyagoda, Madhava Meegaskumbura

Data type: docx

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